Prenatal and early postnatal brain development

The Generation R Study

Sabine J. Roza

Acknowledgements

The Generation R Study is conducted by the Erasmus Medical Center Rotterdam in close collaboration with the Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, the Rotterdam Homecare Foundation and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR), Rotterdam. We gratefully acknowledge the contribution of general practitioners, hospitals, midwives and pharmacies in Rotterdam. The first phase of Generation R was made possible by the Erasmus Medical Center Rotterdam, the Erasmus University Rotterdam; and the Netherlands Organization for Health Research and Development (ZonMw).

The work presented in this thesis was conducted at the Department of Child and Adolescent Psychiatry, Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands and was supported by an additional grant from the Netherlands Organization for Health Research and Development (ZonMw, 'Geestkracht' programme 10.000.1003).

Further financial support for the publication of this thesis was provided by the Department of Child and Adolescent Psychiatry, The Generation R Study, and Janssen-Cilag B.V.

ISBN: 978-90-8559-382-9

Layout and printing: Optima Grafische Communicatie, Rotterdam, The Netherlands
Cover: Mauro Parravicini

Prenatal and Early Postnatal Brain Development

The Generation R Study

Hersenontwikkeling in het prenatale en vroeg postnatale leven

Het Generation R Onderzoek

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
Rector Magnificus

Prof.dr. S.W.J. Lamberts

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
vrijdag 20 juni 2008 om 13.30 uur

door

Sabine Judith Roza

geboren te Rotterdam

Promotiecommissie

Promotoren:	Prof.dr. F.C. Verhulst
	Prof.dr. A. Hofman
Overige leden:	Prof.dr. E.A.P. Steegers
	Prof.dr. R.S. Kahn
	Prof.dr. J.H. Gilmore
Copromotor:	Dr. H. Tiemeier

Paranimfen:	Pauline Jansen
	Stefan Roza

Contents

Manuscripts based on this thesis

Chapter 2.1
Roza SJ, Verburg BO, Jaddoe VWV, Hofman A, Mackenbach JP, Steegers EAP, Witteman JCM, Verhulst FC, Tiemeier H. Effects of maternal smoking in pregnancy on prenatal brain development. The Generation R Study.
Eur J Neurosci 2007 Feb;25(3):611-617

Chapter 2.2
Henrichs J, Schenk JJ, Roza SJ, van den Berg MP, Schmidt HG, Steegers EAP, Hofman A, Jaddoe VWV, Verhulst FC, Tiemeier H. Maternal psychological distress affects fetal growth trajectories. The Generation R Study.
Submitted.

Chapter 2.3
Roza SJ, Jaddoe VWV, Hofman A, Mackenbach JP, Verhulst FC, Tiemeier H. Maternal smoking during pregnancy and child behavior problems. The Generation R Study.
Submitted.

Chapter 2.4
Roza SJ, van Batenburg-Eddes T, Steegers EAP, Jaddoe VWV, Hofman A, Verhulst FC, Tiemeier H. Maternal folate deficiency in early pregnancy is related to a higher risk of behavioral problems in children. The Generation R Study.
Submitted.

Chapter 3.1
Roza SJ, van Lier PAC, Jaddoe VWV, Steegers EAP, Moll HA, Mackenbach JP, Hofman A, Verhulst FC, Tiemeier H. Intrauterine growth and infant temperamental difficulties: the Generation R Study.
J Am Acad Child Adolesc Psychiatry. 2008 Jan 22; [Epub ahead of print]

Chapter 3.2
Roza SJ, Steegers EAP, Verburg BO, Jaddoe VWV, Hofman A, Verhulst FC, Tiemeier H. What is spared by fetal brain sparing? Behavioral problems after fetal circulatory redistribution in the general population. The Generation R Study.
Submitted.

Chapter 4.1

Roza SJ, Govaert PP, Vrooman HA, Lequin MH, Hofman A, Steegers EAP, Moll HA, Jaddoe VWV, Verhulst FC, Tiemeier H. Foetal growth determines cerebral ventricular volume in infants. The Generation R Study.

Neuroimage. 2008 Feb 15;39(4):1491-8

Chapter 4.2

Roza SJ, Govaert PP, Lequin MH, Jaddoe VWV, Moll HA, Steegers EAP, Hofman A, Verhulst FC, Tiemeier H. Cerebral ventricular volume and temperamental difficulties in infancy. The Generation R Study.

J Psychiatry Neurosci. 2008 (in press)

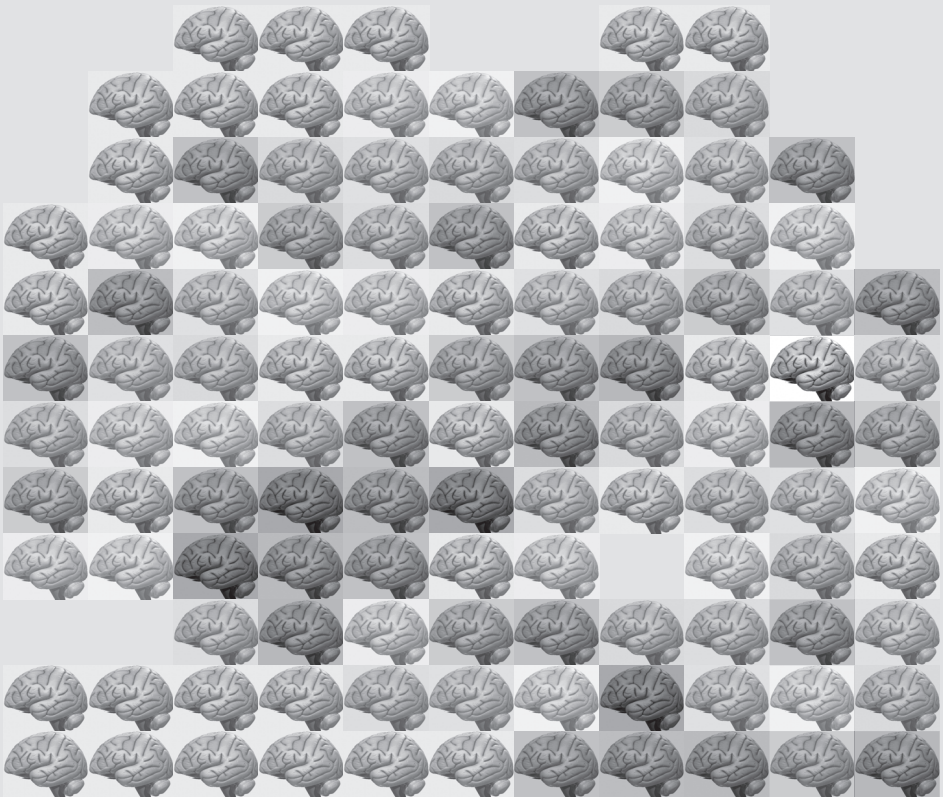
Chapter 4.3

Roza SJ, Govaert PP, de Groot L, Lequin MH, Moll HA, Jaddoe VWV, Hofman A, Verhulst FC, Tiemeier H. Cerebral ventricular volume and neuromotor development in normal infants. The Generation R Study.

Submitted.

Chapter 1

Introduction



Across time, psychiatric disorders were alternately viewed as a biological event, or as a psychological, spiritual or even religious phenomenon [1]. In the 4th century B.C., Hippocrates considered insaneness as a brain disease [2]. The Roman anatomist Galen of Bergama (130-200 A.D.) elaborated on the insights of Hippocrates and hypothesized a close relation between psychological personality and somatic health of a person. A melancholic type, for example, was dominated by black bile, and had a gloomy character [2]. Galen also described the arrangement of the cerebral ventricles [3]. In the Middle Ages, however, mental illness was conceptualized as resulting from punishment from God or possession by the devil. Later, in the Renaissance period, several forms of psychopathology were considered as witchcraft. Although these scientific and occult beliefs existed side by side for a long time [4], the severe stigmatization of mental illness only changed around 1800. At this time, psychiatry became a medical subspecialty [1].

The 19th century was, in psychiatric science, the century of brain research. Brain functions were increasingly localized. The accident of Phineas Gage in 1848, whose frontal lobes were cleaved by an iron rod, showed that higher-order functions of the mind, such as responsibility, personality and compassion, are situated in the brain [3]. Moreover, neuropathology provided insight in common psychiatric disorders like dementia paralytica, which was, in the beginning of the 20th century, affecting about 50% of the patients in psychiatric hospitals. Symptoms of dementia paralytica included manic behavior and grandiosity, as well as dementia and paralysis. Once it was established that these symptoms, coinciding with an overt brain pathology, were actually a late manifestation of syphilis, treatment became available as well [5].

However, many patients who suffered from similar types of psychiatric symptoms did not show any obvious brain pathology [5]. Notwithstanding the unclear neurobiological substrate of these symptoms, one did not hesitate to apply brain surgery in the 1930s. The prefrontal lobotomy of the Portuguese neuropsychiatrist Egas Moniz was a psychosurgical therapy for patients with schizophrenia and severe behavioral disorders, and implied the intersection of all orbitofrontal white matter branches in the frontal lobes [1]. Moniz won the Nobel Prize in 1949 for this treatment, which often had only minimal beneficial effect on agitation of the patient and sometimes had disastrous consequences for the personality of the patients [3]. By 1950, some 20,000 people around the world had been treated this way.

In the 1960s, the evolution in the understanding of neuropharmacology and the identification of neurotransmitters led to the emergence of biological psychiatry [6]. Another discovery at that time was the development of the first systematic non-invasive imaging technique for the study of brain morphology, the computed tomography (CT)-scanner. The first report on structural brain abnormalities in living patients with a severe psychiatric illness was published in 1976 in The Lancet. This study described ventricular enlargement in patients with schizophrenia [7]. The development of both other structural imaging techniques (Magnetic Resonance Imaging, MRI) and diverse functional imaging techniques, have led to

an enormous amount of research on brain changes in psychiatric disorders [8]. In addition to the significant, albeit small and not specific, abnormalities found in brains of patients with schizophrenia, several studies described subtle structural changes in brains of children with attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders [8]. These findings contribute to the hypothesis that several psychiatric disorders are neurodevelopmental in origin [9, 10]. Although the development of the brain continues throughout childhood and adolescence, many alterations in the brains of patients with neurodevelopmental disorders might arise in the prenatal phase.

Indications for the postulated fetal origins of mental health were provided by the Dutch Famine Study. Zena Stein and Mervyn Susser studied the association between maternal undernutrition during pregnancy and adult mental performance in 1972 [11], long before David Barker launched his famous hypothesis that fetal growth restriction due to maternal undernutrition predicts a higher risk of chronic illnesses in adulthood in 1989 [12]. Although Stein and Susser initially found no evidence for an association between undernutrition in fetal life and intelligence [11], their son Ezra Susser and his colleagues reported several years later, with data from the same study, that maternal undernutrition is a risk-factor for schizophrenia [13], antisocial personality disorder [14], and affective disorders [15].

This thesis aims to extend existing knowledge on the prenatal and early neurodevelopmental basis of behavioral and emotional problems. The studies were conducted in the Generation R Study, which offers a unique opportunity to investigate the effects of intrauterine environmental factors on growth and development.

The main aims of this thesis were: 1) to explore the determinants of normal and abnormal brain development in fetal and early postnatal life, 2) to assess whether subtle brain abnormalities in fetuses or young infants increase the risk of problem behavior and neurodevelopmental delays in young children, and 3) to study whether an adverse intrauterine environment is associated with fetal head growth and behavioral problems.

The Generation R Study is a prospective population-based cohort study from fetal life until young adulthood in Rotterdam, the Netherlands [16, 17]. For the present thesis, data from two different samples were used; the Generation R cohort and the Generation R Focus cohort. All mothers who were resident in the study area at their delivery date between April 2002 and January 2006 were eligible for enrolment in the Generation R Study in early pregnancy (gestational age < 18 weeks). In total, 8,880 pregnant women and their children were prenatally enrolled in the study. For postnatal consent, 7620 live born children and their mothers were approached. Differences in the prenatal and postnatal definition of the sample are due to twin pregnancies, withdrawal or lost-to-follow-up during pregnancy, perinatal decease of the child, and exclusion of participants in the pilot phase who lived outside the definite study area (figure 1).

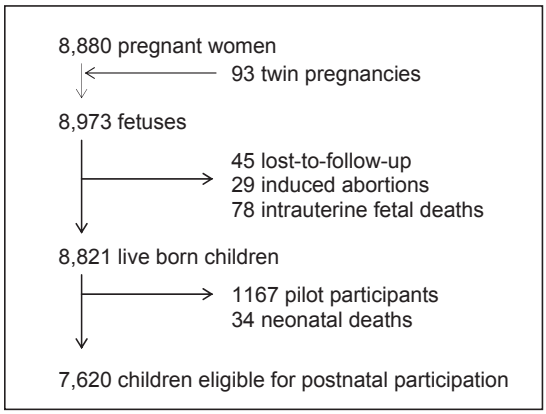


Figure 1 Flow chart

The studies described in chapter 3.2 and chapter 4 were conducted within the Generation R Focus Study. In this subgroup of 1,232 Dutch pregnant women and their children, we performed detailed assessments, such as Doppler ultrasound measurements of the umbilical and cerebral arteries in prenatal life, three-dimensional cranial ultrasound at the age of 6 weeks, and neurodevelopmental examinations. The subgroup is ethnically homogeneous to exclude confounding or effect modification by ethnicity.

Outline

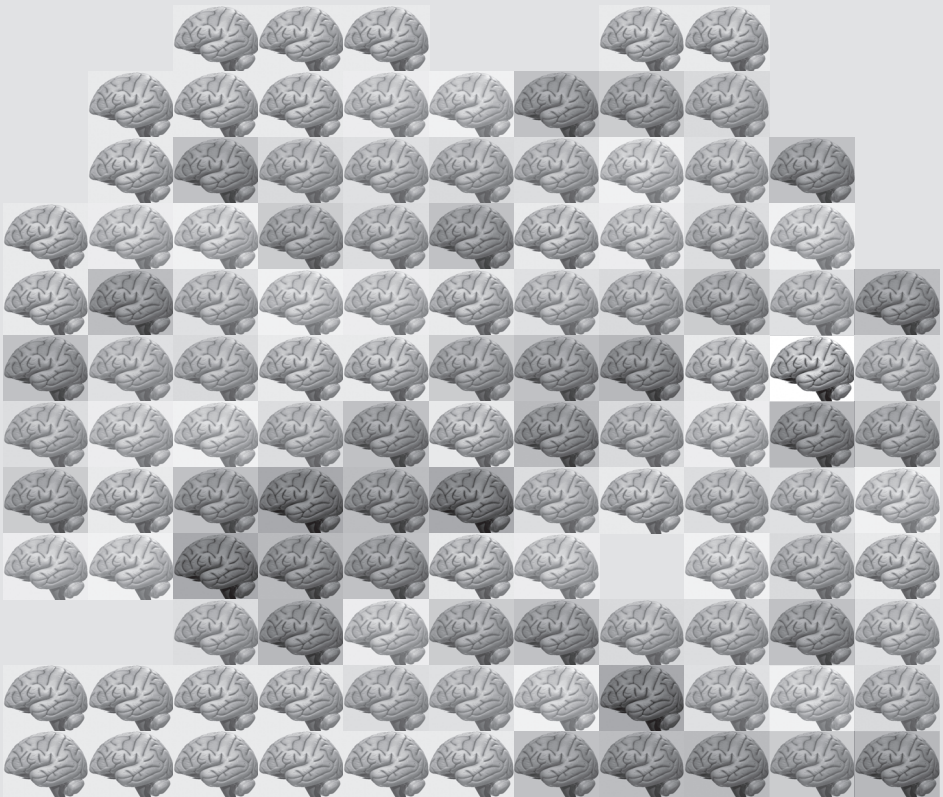
In chapter 2, the effects of intrauterine environmental factors on fetal head growth and behavioral problems are studied. These environmental factors include maternal smoking during pregnancy, maternal distress in pregnancy, and maternal use of folic acid supplements during early pregnancy. Chapter 3 shows whether fetal reactions to an adverse intrauterine environment, such as reduced fetal growth and fetal circulatory redistribution, increase the risk of problem behavior in infancy. In chapter 4, we examine whether a marker of brain maturation, cerebral ventricular volume, is associated with temperamental difficulties and delayed motor development in infancy. Finally, chapter 5 provides a more general discussion of the main findings, and discusses some methodological aspects of the study. This thesis concludes with some implications for clinical practice and future research.

References

1. van Tilburg, W., *Geschiedenis van de psychiatrie*, in *Leerboek psychiatrie*, M.W. Hengeveld and A.J.L.M. van Balkom, Editors. 2005, De Tijdstroom: Utrecht. p. 551 - 567.
2. De Preester, F., *Rede en waanzin. Een korte geschiedenis van de omgang met geesteszieken.* Antenne, 2003: p. 5 - 9.
3. Blakemore, C., *Mechanics of the mind* BBC Reith lectures; 1976. 1977, Cambridge: Cambridge University Press.
4. Merkel, L., *The History of Psychiatry*. 2003.
5. Barondes, S.H., *The biological approach to psychiatry: history and prospects.* J Neurosci, 1990. **10**(6): p. 1707-10.
6. Martin, J.B., *The integration of neurology, psychiatry, and neuroscience in the 21st century.* Am J Psychiatry, 2002. **159**(5): p. 695-704.
7. Johnstone, E.C., et al., *Cerebral ventricular size and cognitive impairment in chronic schizophrenia.* Lancet, 1976. **2**(7992): p. 924-6.
8. Giedd, J.N., et al., *Anatomic brain imaging studies of normal and abnormal brain development in children and adolescents*, in *Developmental Psychopathology (2nd ed.).: Developmental Neuroscience*, D. Cicchetti and D. Cohen, Editors. 2006, Wiley: New York. p. 127-196.
9. Filipek, P.A., *Neuroimaging in the developmental disorders: the state of the science.* J Child Psychol Psychiatry, 1999. **40**(1): p. 113-28.
10. Bradshaw, J.L. and D.M. Sheppard, *The neurodevelopmental frontostriatal disorders: evolutionary adaptiveness and anomalous lateralization.* Brain Lang, 2000. **73**(2): p. 297-320.
11. Stein, Z., et al., *Nutrition and mental performance.* Science, 1972. **178**(62): p. 708-13.
12. Barker, D.J., et al., *Weight in infancy and death from ischaemic heart disease.* Lancet, 1989. **2**(8663): p. 577-80.
13. Susser, E., et al., *Schizophrenia after prenatal famine. Further evidence.* Arch Gen Psychiatry, 1996. **53**(1): p. 25-31.
14. Neugebauer, R., H.W. Hoek, and E. Susser, *Prenatal exposure to wartime famine and development of antisocial personality disorder in early adulthood.* JAMA, 1999. **282**(5): p. 455-62.
15. Brown, A.S., et al., *Further evidence of relation between prenatal famine and major affective disorder.* Am J Psychiatry, 2000. **157**(2): p. 190-5.
16. Hofman, A., et al., *Growth, development and health from early fetal life until young adulthood: the Generation R Study.* Paediatr Perinat Epidemiol, 2004. **18**(1): p. 61-72.
17. Jaddoe, V.W., et al., *The Generation R Study: Design and cohort profile.* Eur J Epidemiol, 2006. **21**(6): p. 475-84.

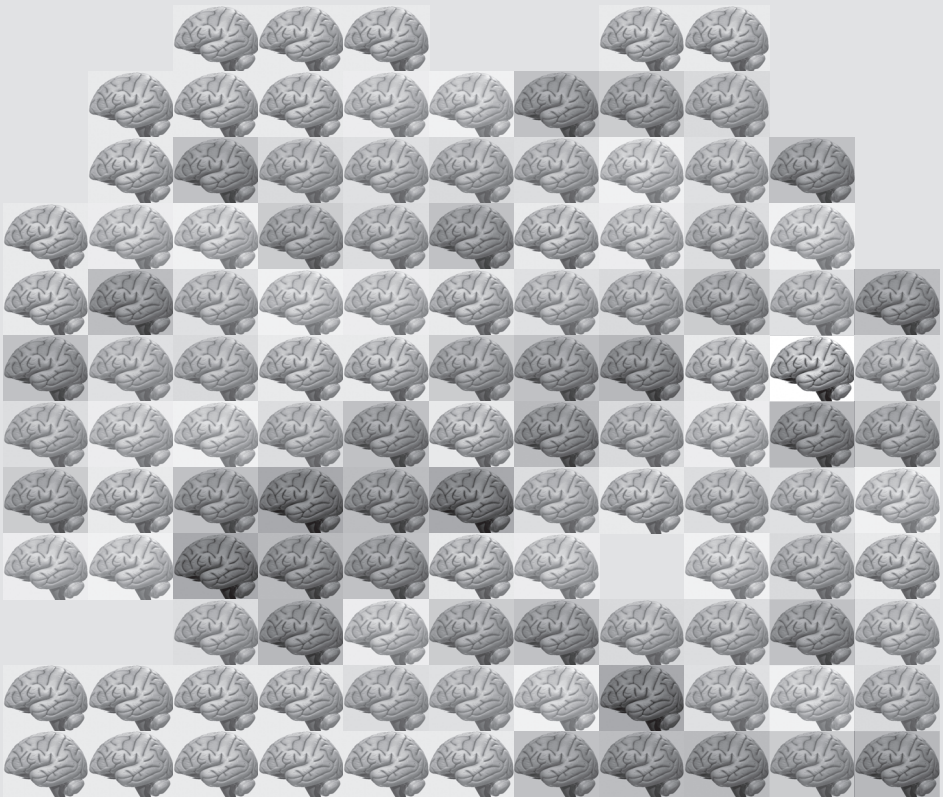
Chapter 2

Intrauterine environmental influences on brain and behavior



2.1

Maternal smoking in pregnancy and prenatal brain development



Abstract

Nicotine, as has been shown in animal studies, is a neuroteratogen, even in concentrations that do not cause growth-retardation. In humans, there is only indirect evidence for negative influences of nicotine on brain development from studies on the association between maternal smoking in pregnancy and behavioral and cognitive development in the offspring. We investigated the associations of maternal smoking in pregnancy with fetal head growth characteristics in 7,042 pregnant women. This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood. Maternal smoking was assessed by questionnaires in early, mid- and late pregnancy. Head circumference, biparietal diameter, transcerebellar diameter and atrial width of lateral ventricle were repeatedly measured by ultrasound. When mothers continued to smoke during pregnancy, fetal head circumference showed a growth reduction of 0.13 mm (95% confidence interval: -0.18;-0.09) per week compared to fetuses of mothers who never smoked during pregnancy. Biparietal diameter of fetuses with smoking mothers grew 0.04 mm (95% confidence interval: -0.05;-0.02) less per week than that of fetuses of non-smoking mothers. Atrial width of lateral ventricle was 0.12 mm (95% confidence interval: -0.22; -0.02) smaller and transcerebellar diameter was 0.08 mm (95% confidence interval: –0.15;-0.00) smaller if mothers smoked, but growth per week of these characteristics was not affected by maternal smoking in pregnancy. In conclusion, continuing to smoke during pregnancy leads to reduced growth of the fetal head. Further research should focus on the causal pathway from prenatal cigarette exposure via brain development to behavioral and cognitive functions.

Introduction

Research has demonstrated the several negative effects of maternal smoking in pregnancy on the developing fetus. Maternal cigarette smoking is an established risk factor for intrauterine growth restriction, perinatal morbidity and mortality and postnatal growth [1, 2]. This is assumed to be the result of fetal hypoxia due to nicotine-induced vasoconstriction, which leads to reduced blood flow to the fetus, and decreased oxygen availability. In addition, carbon monoxide exposure induces higher levels of carboxyhemoglobin, which also leads to intrauterine hypoxia [1, 3]. Several reports described reduced fetal head circumference and biparietal diameter as parameters of total growth restriction in fetuses of smoking mothers [4-8]. So far, animal studies, but rarely human studies, indicate that nicotine directly influences fetal brain development, even in concentrations that do not cause growth retardation [9, 10]. Altered cell proliferation and differentiation due to prenatal exposure of nicotine affects neural cell survival and the development of several neurotransmitter systems [10], including the cholinergic, the dopaminergic and the serotonergic system [11]. In humans, there is mainly indirect evidence for negative influences of nicotine on brain development from studies on the association between maternal cigarette smoking in pregnancy and behavioral and cognitive development in the offspring. The consistent finding of higher rates of behavior problems in children whose mothers smoked during pregnancy is striking, but still difficult to interpret because of numerous confounding environmental and genetic factors [12]. Whether the relationship between maternal smoking in pregnancy and behavioral and cognitive development is mediated by brain deficits, is yet unknown.

To explore the first step in this causal pathway, the present study investigated the associations of maternal smoking in pregnancy with longitudinally measured fetal head growth characteristics. We hypothesized that 1) maternal smoking in pregnancy adversely affects growth of fetal head circumference and biparietal diameter, 2) this effect is independent of total fetal growth restriction and 3) specific brain parameters, i.e. transcerebellar diameter and atrial width of lateral ventricle, are negatively influenced by prenatal nicotine exposure.

Materials and methods

Setting

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. The Generation R Study, designed to identify early environmental and genetic determinants of growth, development and health, has been described previously in detail [13, 14]. Briefly, the cohort includes 9,778 mothers and their children of different ethnicities living in Rotterdam, one of the major cities of the Netherlands.

Enrolment was aimed in early pregnancy (gestational age < 18 weeks) but was possible until birth of the child. Assessments in pregnancy, including physical examinations, ultrasound assessments and questionnaires, were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18-25 weeks) and late pregnancy (gestational age ≥ 25 weeks). The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

Study population

All pregnant women who were resident in the study area at their delivery date from April 2002 until January 2006 were invited to participate. Of the total of 9,778 mothers (response rate 61%), 91% (n=8,880) was enrolled in pregnancy [14] and was eligible for present analyses. Mothers without information on smoking habits in pregnancy on the basis of the three questionnaires were excluded from the present study (4.9%, n=433). Mothers with twin pregnancies (n=90) were excluded since growth potentials for individual fetuses in multiple pregnancies are not comparable to singleton pregnancies. Of the remaining 8,357 mothers, 4.4% (n=364) had only one ultrasound assessment during pregnancy. 1,852 (22.2%) mothers had two ultrasound assessments, while most participating mothers (n=6,141; 73.5%) had three ultrasound assessments. In the present study, we restricted analyses to mothers with measurements in early, mid- and late pregnancy for head circumference (n = 5,180) and biparietal diameter (n = 5,501). Since transcerebellar diameter and atrial width of lateral ventricle can only be measured reliably from mid-pregnancy onwards, we restricted the analyses of the association between maternal smoking in pregnancy and these parameters to two measurements in mid- and in late pregnancy. Analyses were based on 5,675 subjects for transcerebellar diameter and 3,071 subjects for atrial width of lateral ventricle. Overall, 7,042 mothers were included in one or more analyses.

Maternal smoking in pregnancy

Information about maternal smoking was obtained by postal questionnaires in early, mid- and late pregnancy. Maternal smoking at enrolment was assessed in the first questionnaire by asking whether mother smoked in pregnancy (no, until pregnancy was known, continued during pregnancy). In the second and third questionnaire, mothers were asked whether they smoked in the past 2 months (yes, no) in mid- and late pregnancy, respectively. Maternal smoking during pregnancy was categorized on the basis of all three questionnaires into 'no', 'until pregnancy was known' and 'continued during pregnancy'. Mothers who reported in the first questionnaire to have smoked until pregnancy was known but reported to smoke in

the second or third questionnaire, were classified as 'continued smoking during pregnancy'. Similarly, mothers who reported not to smoke in the first questionnaire but acknowledged to smoke in the second or third questionnaire were classified as 'continued during pregnancy'. When information was missing on maternal smoking at enrolment, information from the second and / or third questionnaire was used to classify mothers into 'no smoking in pregnancy' or 'continued smoking during pregnancy'.

Fetal ultrasound examinations

Sonographers carried out fetal ultrasound examinations at the visits to the research centers in early, mid- and late pregnancy. Most assessments (88%) were performed at the Generation R research center in Rotterdam, the remaining were carried out at the five collaborating hospitals. The fetal ultrasound examinations were used for both establishing gestational age and assessing fetal growth characteristics. Crown-rump length was used for pregnancy dating until a gestational age of 12 weeks and biparietal diameter was used for pregnancy dating thereafter. In additional analyses, we used history of last menstrual period to date pregnancy. To avoid bias from irregularity of menstrual cycles or recall problems, we only included ultrasound measurements when gestational duration on the basis of last menstrual period dating and gestational duration on the basis of ultrasound dating differed less than three weeks.

The median (95% range) gestational age for the fetal ultrasound examinations in early, mid- and late pregnancy was 13.1 (9.1 – 17.5) weeks, 20.5 (18.4 – 23.5) weeks and 30.3 (27.2 – 33.0) weeks. Online measurements used for the present study included head circumference, biparietal diameter, transcerebellar diameter and atrial width of lateral ventricles and were done using standardized techniques. The biparietal diameter represents the widest diameter of the fetal head in a transverse plane, measured outer to outer of the fetal skull, perpendicular to the midline. The head circumference was measured at the level of the biparietal diameter and represents the outer perimeter of the fetal skull. The atrial width of the lateral ventricle is the widest diameter of the atrium of one of the lateral ventricles that can be measured in an axial plane. A slightly caudal rotated axial plane was used for measuring the transcerebellar diameter as the widest diameter across both hemispheres of the cerebellum. Sonographers were blinded to smoking status of the pregnant women. High intraobserver and interobserver reproducibility for biparietal diameter, head circumference and atrial width of lateral ventricle have been reported [15, 16].

The intra- and interobserver reliability of fetal biometry measurements in early pregnancy within the Generation R Study is good (intraclass correlation coefficients for head circumference 0.995 (intraobserver) and 0.988 (interobserver) and for biparietal diameter 0.995 (intraobserver) and 0.994 (interobserver) with coefficients of variation between 1.8 and 3.8%).

Covariates

The following variables were considered as possible confounders: maternal age, fetal gender, maternal height, maternal body mass index, maternal educational level, maternal ethnicity, parity, maternal alcohol consumption, maternal prenatal anxiety and maternal prenatal depression. Maternal age and maternal anthropometrics were assessed at enrolment in one of the research centers. Height and weight were measured without shoes and heavy clothing and body mass index was calculated from height and weight (weight / height^2). Information on maternal educational level, maternal ethnicity and parity was obtained by the first questionnaire at enrolment in the study. Maternal alcohol consumption was assessed in early, mid- and late pregnancy by questionnaire and categorized into 'no alcohol use', 'alcohol use until pregnancy was known' and 'continued using alcohol during pregnancy'. Maternal anxiety and depression were assessed in mid-pregnancy using two scales of the Brief Symptom Inventory (BSI), a validated self-report questionnaire with 53 items scored on a 5-point scale [17, 18]. Fetal gender was obtained from midwife and hospital registries at birth.

Data analysis

The associations between maternal smoking habits during pregnancy and repeatedly measured brain parameters (head circumference, biparietal diameter, transcerebellar diameter and atrial width of lateral ventricle) were analyzed using longitudinal multilevel analysis [19] to account for the dependency between measurements in the same subject. First, the best fitting model with the outcome as a function of gestational age was constructed using fractional polynomials [20]. Second, maternal smoking as main determinant was brought into the model. The final curve was fitted with random effects for both intercept and gestational age. The interaction term of maternal smoking with gestational age was included in the model to compare the slope of the curves between the different smoking categories. When this interaction term did not result in a significant improvement of the model (evaluated by comparing the –2 log likelihood of the model with the interaction term to the –2 log likelihood of the model without the interaction term), the term was left out in further analyses.

The best fitting models were the following:

Head circumference = $\beta_0 + [\beta_1 * \text{smoking}] + [\beta_2 * \text{gestational age}^2] + [\beta_3 * \text{gestational age}^2 * \ln(\text{gestational age})] + [\beta_4 * \text{smoking} * \text{gestational age}]$.

Biparietal diameter = $\beta_0 + [\beta_1 * \text{smoking}] + [\beta_2 * \text{gestational age}] + [\beta_3 * \text{gestational age}^3] + [\beta_4 * \text{smoking} * \text{gestational age}]$.

Transcerebellar diameter = $\beta_0 + [\beta_1 * \text{smoking}] + [\beta_2 * \text{gestational age}^2]$.

Atrial width of lateral ventricle = $\beta_0 + [\beta_1 * \text{smoking}] + [\beta_2 * \text{gestational age}]$.

In these models, 'β_0 + [β_1 x smoking]' reflects the intercept and the terms including 'β_2' and 'β_3' reflect the slope of growth per week. Terms including 'β_4' reflect the differences in growth of each characteristic between the maternal smoking categories.

Models were based on 15,540 observations for head circumference, 16,503 observations for biparietal diameter, 11,350 observations for transcerebellar diameter and 6,142 observations for atrial width of lateral ventricle. All models were adjusted for life style and socio-economic status related confounders (maternal body mass index and educational level) and other known determinants of fetal growth (maternal age, height, ethnicity, parity and fetal gender). Maternal alcohol use, maternal prenatal anxiety and maternal prenatal depression did not significantly improve the models and did not change the regression coefficients for maternal smoking. These covariates were therefore excluded in final analyses. History of last menstrual period of reasonable quality, i.e. less than 3 weeks difference from ultrasound-defined age, was available for 4,592 subjects. A p-value of 0.05 was taken to indicate statistical significance. Statistical analyses were carried out using SAS v.8.2 (Stata Corporation, College Station, TX, USA), including the Proc Mixed module for longitudinal multilevel analysis.

Results

Characteristics of pregnant women per smoking category are presented in table 1. Of all mothers, 7.7% (n=545) reported to smoke until pregnancy was known and 17.0 % (n=1,199) continued smoking during pregnancy. Mothers who continued smoking in pregnancy were younger, lower educated and more often used alcohol during pregnancy compared to mothers who never smoked during pregnancy. Anxiety and depression scores were higher in mothers who continued to smoke during pregnancy than in non-smoking mothers. More Turkish women (30.3%) continued smoking during pregnancy compared to Dutch women (16.2%), while only few Moroccan mothers (5.4%) smoked during pregnancy.

Table 2 presents mean values for head circumference, biparietal diameter, transcerebellar diameter and atrial width of lateral ventricle at the median gestational ages in early, mid- and late pregnancy. There was a strong significant correlation between head circumference and biparietal diameter (r=0.7) measured at the median gestational age in late pregnancy. Head circumference (r=0.3) and biparietal diameter(r=0.3) were moderately correlated to transcerebellar diameter, while the correlation of the atrial width of lateral ventricle with head circumference (r=0.2) and biparietal diameter (r=0.2) was low, but significant. Transcerebellar diameter was not significantly related to atrial width of lateral ventricle.

The associations between maternal smoking habits in pregnancy and longitudinally measured growth and developmental brain parameters are presented in table 3. Adjustment for the covariates maternal age, body mass index, height, educational level, ethnicity, parity and fetal gender changed the effect of maternal smoking by 1.2 – 14.1 %. In the models with head

Table 1. Maternal characteristics

	Groups of mothers in relation to smoking		
	Non-smoking (n=5298)	Quit smoking when pregnancy known (n=545)	Continued smoking during pregnancy (n=1199)
Age (years)	30.1 ± 5.0	29.4 ± 5.0*	28.3 ± 5.7**
Height (cm)	167.3 ± 7.4	168.5 ± 7.0*	166.9 ± 7.1
Body mass index (kg/m^2)	24.6 ± 4.3	24.5 ± 4.4*	24.9 ± 4.6*
Educational level			
Primary level (%)	9.7	11.7	20.9
Secondary level (%)	40.7	43.3	60.0
Higher education (%)	49.4	45.0	19.0
X^2 (and d.f.) vs. non-smoking mothers	-	4.6 (2)	343 (2)**
Ethnicity			
Dutch (%)	51.6	55.5	49.4
Turkish (%)	7.3	7.0	15.5
Moroccan (%)	7.5	1.1	1.9
Cape Verdean (%)	3.8	4.6	5.4
Antillean (%)	3.1	2.2	4.2
Surinamese (%)	8.1	11.3	10.1
Other Western (%)	8.8	9.6	7.9
Other non-Western (%)	9.8	8.7	5.6
X^2 (and d.f.) vs. non-smoking mothers	-	39 (7)**	141 (7)**
Parity (% nulli)	55.4	68.2**	54.7
Alcohol use in pregnancy			
No (%)	52.3	25.2	46.5
Until pregnancy was known (%)	11.3	27.2	11.8
Continued during pregnancy (%)	36.4	47.6	41.6
X^2 (and d.f.) vs. non-smoking mothers	-	188 (2)**	13.6 (2)**
Depression score	0.20 ± 0.4	0.21 ± 0.5	0.42 ± 0.7**
Anxiety score	0.26 ± 0.4	0.30 ± 0.5	0.44 ± 0.6**

Values are means ± SD for continuous variables and percentages for categorical variables. * $p < 0.01$, ** $p < 0.001$; ANOVA or Kruskal-Wallis with post hoc comparisons for continuous variables, X^2-tests for categorical covariates, vs. non-smoking mothers.

circumference and biparietal diameter, both the separate smoking variables and the interaction terms of smoking with gestational age significantly contributed to the model. The main (positive) effect of continued maternal smoking on head circumference (regression coefficient 1.89; 95% confidence interval: 1.12; 2.67) should not be interpreted because the interaction effect was included in the model. The interaction effect of continued maternal smoking with gestational age shows that fetal head circumference in mothers who continued smoking during pregnancy grew 0.13 mm less per week compared to fetuses of mothers who never smoked in pregnancy. Figure 1 shows the estimated differences in fetal head circumference of mothers who continued smoking during pregnancy and mothers who quit smoking when pregnancy was known compared to fetal head circumference of mothers who never smoked during pregnancy. This figure shows differences instead of the crude effects of maternal

Table 2. Head circumference, biparietal diameter, transcerebellar diameter, and atrial width of lateral ventricle at median gestational age in early, mid- and late pregnancy

	Early pregnancy (median 13.1 weeks)	Mid-pregnancy (median 20.4 weeks)	Late pregnancy (median 30.4 weeks)
Head circumference (mm)	82.3 ± 5.3	176.4 ± 6.6	284.6 ± 9.3
Biparietal diameter (mm)	23.6 ± 1.4	49.8 ± 2.1	80.3 ± 3.1
Transcerebellar diameter (mm)	-	20.7 ± 1.0	37.7 ± 1.8
Atrial width of lateral ventricle (mm)	-	5.7 ± 1.1	4.9 ± 1.7

Values are means ± SD.

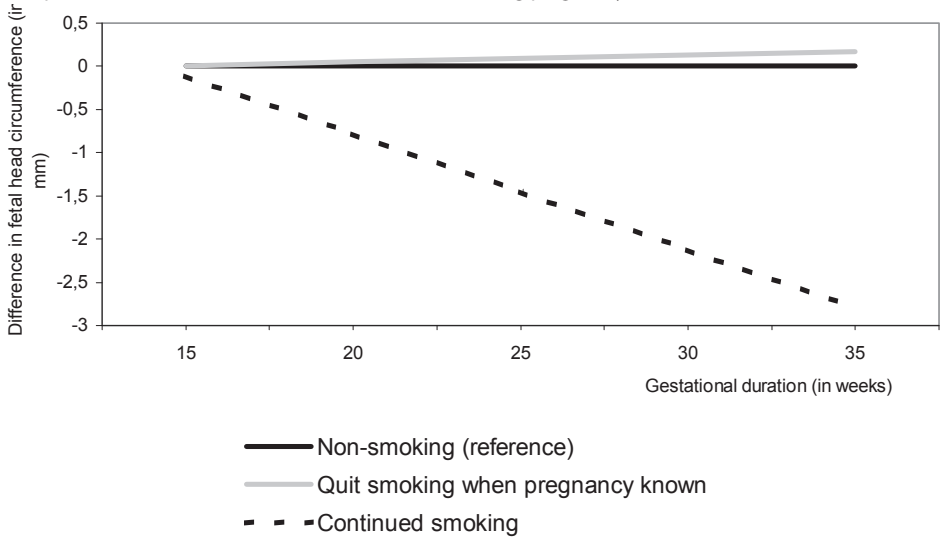
Table 3. Regression coefficients: associations between maternal smoking habits in pregnancy and repeatedly measured head and brain parameters

	Regression coefficients (and 95% confidence intervals)			
	Head circumference	Biparietal diameter	Transcerebellar diameter	Atrial width of lateral ventricle
Non-smoking	*Reference*	*Reference*	*Reference*	*Reference*
Quit smoking	-0.12 (-1.15; 0.92)	-0.29 (-0.60; 0.03)	0.01 (-0.09; 0.11)	0.01 (-0.12; 0.14)
Continued smoking	1.89 (1.12; 2.67)**	0.53 (0.30; 0.76)**	-0.08 (-0.15;-0.00)	-0.12 (-0.22;-0.02)*
GA	5.38 (4.36; 7.30)**	3.98 (3.97; 4.00)	-0.12 (-0.22;-0.01)*	-0.09 (-0.09;-0.08)**
GA^2	1.09 (0.93; 1.25)**	-	0.04 (0.03; 0.04)*	-
GA^3	-	-0.00 (-0.00;-0.00)**	-	-
GA^2 x ln(GA)	-0.27 (-0.30;-0.23)**	-	-	-
GA x non-smoking	*Reference*	*Reference*	-	-
GA x quit smoking	0.01 (-0.05; 0.07)	0.02 (-0.00; 0.04)	-	-
GA x continued smoking	-0.13 (-0.18;-0.09)**	-0.04 (-0.05;-0.02)**	-	-

Models were constructed using fractional polynomials for gestational age. GA, gestational age. Values are regression coefficients and 95% confidence intervals (95% CI) relative to the non-smoking group. All values are adjusted for maternal age, body mass index, height, educational level, ethnicity, parity, and fetal gender.
* $p < 0.05$, ** $p < 0.01$.

smoking on head circumference since the effect sizes were small (max. 2.8 mm difference) and the growth rate was high (from 80 to 300 mm during pregnancy). Total effect size of continued smoking during pregnancy on fetal head circumference varied from 0.11% (at 15 weeks of gestational age) to 0.87% (at 35 weeks of gestational age). When we additionally adjusted the model for abdominal circumference measured at the same gestational age, the effect of continued maternal smoking on fetal head circumference became smaller (0.09 mm / week growth reduction; 95% confidence interval: –0.13; -0.04), but remained statistically significant. In fetuses of smoking mothers biparietal diameter grew 0.04 mm less per week than that of fetuses of non-smoking mothers. The effect size of continued smoking varied from 0.10% - 0.80% for biparietal diameter over time. When fetuses dated on biparietal diameter were left out of the analyses, the effect of maternal smoking on growth of biparietal diameter did not materially change (data not shown). Continued smoking in pregnancy significantly decreased the fetal transcerebellar diameter (0.08 mm smaller) and fetal atrial width of the lateral ventricle (0.11 mm smaller) compared to fetuses of mothers who never smoked during pregnancy. The interaction term of smoking with gestational age did not result in a better fit

Figure 1. Estimated differences of fetal head circumference due to maternal smoking in pregnancy compared to fetuses of mothers who never smoked during pregnancy.



Values are estimated differences in fetal head circumferences based on the linear mixed models, adjusted for maternal age, body mass index, height, educational level, ethnicity, parity and fetal gender.

in the models of transcerebellar diameter or atrial width of lateral ventricle, i.e. the slope of the growth curve during pregnancy did not differ between the three smoking groups.

Finally, we analyzed our data using last menstrual period for pregnancy dating. Head circumference grew 0.10 mm less per week (95% confidence interval: –0.16;-0.05) in fetuses of continued smoking mothers compared to fetuses of non-smoking mothers. Fetal biparietal diameter, when exposed to maternal smoking throughout pregnancy, showed 0.03 mm per week decreased growth (95% confidence interval: –0.04;-0.01). Transcerebellar diameter was significantly smaller in fetuses of mothers who continued smoking in pregnancy (-0.14 mm, 95% confidence interval: -0.26;-0.01) compared to fetuses of mothers who never smoked in pregnancy. Atrial width of lateral ventricle was not significantly affected by maternal smoking in pregnancy in the analyses based on last menstrual period dating (0.02 mm, 95% confidence interval: –0.11;0.14).

Discussion

This study showed that maternal smoking in pregnancy is associated with reduced growth of the fetal head. Small but highly significant associations were found for head circumference and biparietal diameter. Maternal smoking did also result in smaller atrial width of lateral ventricle and smaller transcerebellar diameter but the differences between fetuses of smoking and non-smoking mothers remained constant throughout pregnancy.

Three different mechanisms to explain the untoward effects of maternal smoking in pregnancy on neurobehavioral development have been proposed. First, cigarette smoking leads to intrauterine growth restriction due to fetal hypoxia [1, 4, 5]. Research, which initially focused on decreased birth weight, now mostly utilizes ultrasound to measure fetal growth characteristics. Cigarette exposure seems to cause a more general, symmetrical growth restriction including decreased head circumference [6, 7, 21, 22], while brain maturation is usually maintained in mild fetal growth restriction (the 'brain sparing effect'). Our study also showed reduced growth of the fetal head circumference and biparietal diameter, even after controlling for the effects of maternal smoking on abdominal circumference. Growth of the transverse cerebellum diameter was not affected by cigarette exposure in our study, which is consistent with the fact that the cerebellum is the least affected in growth restriction [22].

The second mechanism proposed is the direct effect of nicotine on the developing brain, which has mainly been shown in animal studies. Cell replication and differentiation, the most prominent features in fetal brain development, are controlled partly by neurotransmitter-induced stimulation. Doses of nicotine below the threshold that causes growth retardation, induct premature stimulation of this receptor-mediated process, which results in cell damage, cell loss and synaptic dysfunction [23]. Increased cell death has been demonstrated in both cortex and cerebellum [11], whereas changes in expression of nicotinic and muscarinic acetylcholine receptors were seen in human brainstem and cerebellum in the first trimester [16]. The long-term effects of functional alteration of nicotinic acetylcholine receptors in the human brain remain unclear. No studies examined these subtle changes or their relation to developmental outcomes in living human brains. In the present study, we found structural alterations in the cerebellum by prenatal cigarette exposure, which indicates that nicotine induces cell loss in this region. To our knowledge, effects of nicotine on the ventricular system have not been described in animal or human studies until now. Atrial width of the lateral ventricle, a measurement for verifying the state of the ventricular system, provides information on the growth of the cerebral hemispheres during mid- and late pregnancy. In contrast to an earlier report on 100 healthy fetuses [12, 24, 25], our study on more than 5,000 fetuses showed a decrease of the mean lateral ventricle atrium diameter throughout gestation. Most likely, the narrowing of the ventricles is due to growth of the cerebral hemispheres. Cigarette exposure did not influence the narrowing of these cavities.

A third explanation for the effect of prenatal maternal smoking on neurodevelopment in the offspring are the epiphenomena of smoking, e.g. parental psychopathology, co-abuse of other substances, poor prenatal care, dietary restriction and low socio-economic status. The large amount of studies on associations between maternal smoking during pregnancy and subsequent mental health problems in offspring has provided strikingly consistent evidence for an etiologic role for prenatal cigarette exposure in the onset of conduct disorder, antisocial behavior and attention deficit hyperactivity disorder as well as subtle intellectual decrements and neurocognitive impairments, e.g. [12, 24, 25]. However, the interpretation

of these findings still leads to discussion. Some reviewers have pointed out that the found effects, especially those on cognitive functioning and academic achievement, attenuate completely after control for a variety of co-varying influences [26]. In line with other studies [27], we found increased scores on depression and anxiety in mothers who continued to smoke during pregnancy. These mothers were also lower educated than non-smoking pregnant women. It is clear that maternal smoking in pregnancy frequently occurs in the context of other factors that place the child at increased developmental risk, and it remains a challenge to fully control for these confounding variables.

It is tempting to speculate that reduced growth of the head circumference and biparietal diameter is one of the mediators in the relationship between maternal smoking in pregnancy and behavior in the offspring. The neurobehavioral consequences of subnormal head circumference are well-known: in (very) low birth weight children, this indicator of brain volume was negatively associated with cognitive function and neuropsychological abilities at early school age [28]. Reductions in brain volume, as measured by structural magnetic resonance imaging studies, were associated with poorer cognitive outcome [29]. Furthermore, several MRI investigations in clinical samples revealed information on alterations in total cerebral volume in ADHD, schizophrenia and autism. Both children with ADHD and children with schizophrenia seem to have decreased total brain volumes [29]. In addition, reduced cerebellar volumes and decreased growth of the lateral ventricular system have been found in children and adolescents with ADHD [30]. Our data suggest that the structural changes induced by prenatal exposure to tobacco smoke are similar to the structural changes found in ADHD. However, whether the morphological alterations in this and other child psychiatric disorders are due to prenatal nicotine exposure or caused by genetic and other environmental influences cannot be inferred from our data.

Strengths of this study are the large number of participating pregnant women, its prospective population-based design, the repeated measurements of fetal head growth parameters during pregnancy and the information on numerous potential confounding variables. However, some methodological issues need to be considered. Firstly, the specific brain parameters transcerebellar diameter and atrial width of lateral ventricle were not measured in all participating pregnant women. This was mainly due to the fact that these parameters were added to the study protocol during data collection. These missing outcomes are assumed to be random across participants. However, we cannot rule out non-random effects as ultrasound data probably were more complete in healthier, Dutch-speaking and higher-educated participants who also might have different smoking patterns. Secondly, information about maternal smoking habits during pregnancy was collected by postal questionnaires. Using self-reports may have introduced misclassification mainly due to underreporting of cigarette consumption, which could lead to underestimation of the effects. Thirdly, the main disadvantage of using fetal ultrasound examinations for pregnancy dating is that growth variation before the first measurement is set to zero. This makes it impossible to notice effects of ma-

ternal smoking on fetal growth in early pregnancy. Furthermore, Henriksen *et al*. [31] showed that this method can distort estimates of the effects of maternal smoking in pregnancy on preterm and postterm delivery. However, the use of last menstrual period for pregnancy dating can be biased by recall problems, irregularity of menstrual cycles or contraceptive use. Thus, we performed analyses with both dating methods and found very small differences in results. The similar results indicate that the found effects of maternal smoking in pregnancy on fetal head growth characteristics are not biased by the method of pregnancy dating. Finally, despite our efforts to control for several determinants of fetal growth and life-style related confounders, it cannot be ruled out that the associations are confounded by diet-related determinants or other, unknown, factors.

In conclusion, prenatal cigarette exposure leads to reduced growth of the fetal brain. Growth of the cerebellum or ventricular system was not affected by maternal smoking, but molecular alterations and changes in neurotransmitter systems can still be present. The effects of maternal smoking in pregnancy were significant, even after controlling for numerous important confounders. One cannot conclude from our results which components in tobacco smoke induce the structural changes within the developing fetus. We recommend clinical and public health strategies aimed at the primary and secondary prevention of prenatal tobacco exposure of children. In this respect, it is important to know that quitting smoking as soon as pregnancy is known positively affects fetal growth and development. Primary caregivers should inform young women about the (long-term) consequences of smoking during pregnancy. Future research should comprise large prospective studies, which relate the structural and functional changes in the human fetal brain after prenatal nicotine exposure to children's behavioral and cognitive development.

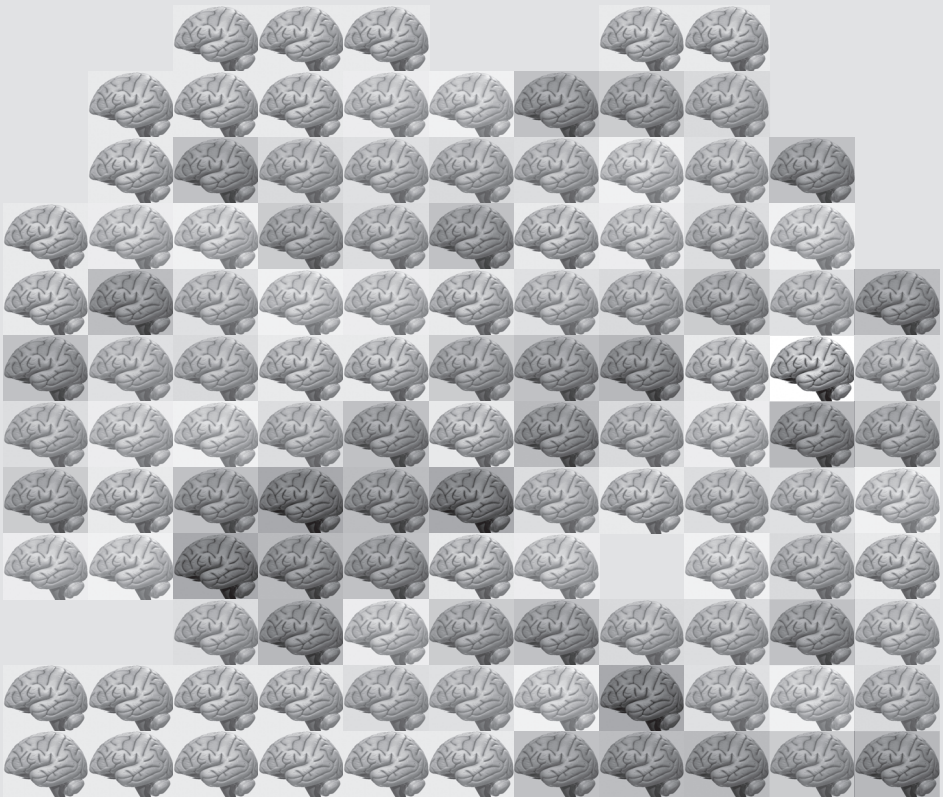
References

1. Abel, E.L., *Smoking during pregnancy: a review of effects on growth and development of offspring.* Hum Biol, 1980. **52**(4): p. 593-625.
2. Lieberman, E., et al., *Low birthweight at term and the timing of fetal exposure to maternal smoking.* Am J Public Health, 1994. **84**(7): p. 1127-31.
3. Lehtovirta, P. and M. Forss, *The acute effect of smoking on intervillous blood flow of the placenta.* Br J Obstet Gynaecol, 1978. **85**(10): p. 729-31.
4. Jeanty, P., et al., *Sonographic detection of smoking-related decreased fetal growth.* J Ultrasound Med, 1987. **6**(1): p. 13-8.
5. Newnham, J.P., et al., *Effects of maternal cigarette smoking on ultrasonic measurements of fetal growth and on Doppler flow velocity waveforms.* Early Hum Dev, 1990. **24**(1): p. 23-36.
6. Vik, T., et al., *Pre- and post-natal growth in children of women who smoked in pregnancy.* Early Hum Dev, 1996. **45**(3): p. 245-55.
7. Zaren, B., G. Lindmark, and L. Bakketeig, *Maternal smoking affects fetal growth more in the male fetus.* Paediatr Perinat Epidemiol, 2000. **14**(2): p. 118-26.
8. Jaddoe, V.W.V., et al., *Maternal smoking and fetal growth characteristics in different periods of pregnancy. The Generation R Study.* Am J Epidemiol, 2007. **in press**.
9. Law, K.L., et al., *Smoking during pregnancy and newborn neurobehavior.* Pediatrics, 2003. **111**(6 Pt 1): p. 1318-23.
10. Slotkin, T.A., *Fetal nicotine or cocaine exposure: which one is worse?* J Pharmacol Exp Ther, 1998. **285**(3): p. 931-45.
11. Falk, L., et al., *Smoking during early pregnancy affects the expression pattern of both nicotinic and muscarinic acetylcholine receptors in human first trimester brainstem and cerebellum.* Neuroscience, 2005. **132**(2): p. 389-97.
12. Ernst, M., E.T. Moolchan, and M.L. Robinson, *Behavioral and neural consequences of prenatal exposure to nicotine.* J Am Acad Child Adolesc Psychiatry, 2001. **40**(6): p. 630-41.
13. Hofman, A., et al., *Growth, development and health from early fetal life until young adulthood: the Generation R Study.* Paediatr Perinat Epidemiol, 2004. **18**(1): p. 61-72.
14. Jaddoe, V.W., et al., *The Generation R Study: Design and cohort profile.* Eur J Epidemiol, 2006. **21**(6): p. 475-84.
15. Perni, S.C., et al., *Intraobserver and interobserver reproducibility of fetal biometry.* Ultrasound Obstet Gynecol, 2004. **24**(6): p. 654-8.
16. Cardoza, J.D., R.B. Goldstein, and R.A. Filly, *Exclusion of fetal ventriculomegaly with a single measurement: the width of the lateral ventricular atrium.* Radiology, 1988. **169**(3): p. 711-4.
17. Derogatis, L.R. and N. Melisaratos, *The Brief Symptom Inventory: an introductory report.* Psychol Med, 1983. **13**(3): p. 595-605.
18. de Beurs, E., *Brief Symptom Inventory, handleiding.* 2004: Leiden, the Netherlands.
19. Twisk, J.W.R., *Applied Multilevel Analysis. A practical Guide*. 2006, Cambridge, UK: University Press.
20. Royston, P., G. Ambler, and W. Sauerbrei, *The use of fractional polynomials to model continuous risk variables in epidemiology.* Int J Epidemiol, 1999. **28**(5): p. 964-74.
21. Kallen, K., *Maternal smoking during pregnancy and infant head circumference at birth.* Early Hum Dev, 2000. **58**(3): p. 197-204.
22. Campbell, W.A., et al., *Use of the transverse cerebellar diameter/abdominal circumference ratio in pregnancies at risk for intrauterine growth retardation.* J Clin Ultrasound, 1994. **22**(8): p. 497-502.
23. Slotkin, T.A., *Developmental cholinotoxicants: nicotine and chlorpyrifos.* Environ Health Perspect, 1999. **107 Suppl 1**: p. 71-80.
24. Weitzman, M., et al., *The effects of tobacco exposure on children's behavioral and cognitive functioning: implications for clinical and public health policy and future research.* Neurotoxicol Teratol, 2002. **24**(3): p. 397-406.

25. Linnet, K.M., et al., *Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: review of the current evidence.* Am J Psychiatry, 2003. **160**(6): p. 1028-40.
26. Breslau, N., M. Kilbey, and P. Andreski, *Nicotine dependence, major depression, and anxiety in young adults.* Arch Gen Psychiatry, 1991. **48**(12): p. 1069-74.
27. Peterson, J., et al., *Subnormal head circumference in very low birth weight children: neonatal correlates and school-age consequences.* Early Hum Dev, 2006. **82**(5): p. 325-34.
28. Peterson, B.S., et al., *Regional brain volume abnormalities and long-term cognitive outcome in preterm infants.* Jama, 2000. **284**(15): p. 1939-47.
29. Eliez, S. and A.L. Reiss, *MRI neuroimaging of childhood psychiatric disorders: a selective review.* J Child Psychol Psychiatry, 2000. **41**(6): p. 679-94.
30. Castellanos, F.X., et al., *Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder.* Arch Gen Psychiatry, 1996. **53**(7): p. 607-16.
31. Henriksen, T.B., et al., Bias in studies of preterm and postterm delivery due to ultrasound assessment of gestational age. *Epidemiology*, 1995. **6**(5): p. 533-7.

2.2

Maternal psychological distress and fetal growth trajectories



Abstract

Context: Previous research suggests that maternal psychological distress during pregnancy leads to adverse birth outcomes, such as low birth weight and preterm delivery.

Objective: To investigate whether maternal psychological distress affects fetal size and growth from mid-pregnancy until birth.

Design: A prenatally recruited multi-ethnic population-based cohort study.

Setting: The Generation R Study cohort of pregnant women in Rotterdam, The Netherlands.

Participants: 6,313 mothers, who were assessed with the Brief Symptom Inventory and the Family Assessment to measure symptoms of anxiety and depression and family stress at 20 weeks pregnancy.

Main Outcome Measures: Fetal ultrasound measurements were performed in mid- and late pregnancy and included head and abdominal circumference, and femur length. Estimated fetal weight was calculated using the formula by Hadlock. Birth weight was obtained from medical records.

Results: Affective symptoms during pregnancy led to a reduced growth of fetal head and abdominal circumference and family stress was negatively related to growth trajectories of the fetal head and femur after adjustment for potential confounders. All forms of maternal distress were related to fetal weight gain during pregnancy. A 1 standard deviation increase of depressive symptoms reduced fetal weight gain by 1.36 grams (95% CI: -1.93; -0.79, $p < .001$) per week. However, maternal distress was not associated with fetal size in mid- and late pregnancy. A 1 standard deviation increase of maternal anxious symptoms during pregnancy resulted in a 13.02 grams (95% CI: -24.59; -1.45, $p = .027$) lower birth weight. No other form of maternal distress was linked to birth weight.

Conclusions: The study suggests that fetal growth from mid-pregnancy onwards can be affected by different forms of maternal distress. Future work should address the biological mechanisms underlying the association of maternal distress with fetal development and focus on the effects of reducing psychological distress in pregnancy.

Introduction

The belief that the emotional state of the pregnant woman affects the development of the fetus is ancient and found in all cultures [1]. Animal research shows that exposure to stress in utero is related to lower fetal and birth weight of the offspring [2-4]. Recent human studies have demonstrated that maternal prenatal depression, anxiety and stress are associated with higher rates of spontaneous abortion [5] and preeclampsia [6]. Furthermore, maternal psychological distress in pregnancy is related to an increased risk of preterm delivery, low birth weight, and a possibly smaller head circumference at birth [7-15].

Although several studies have investigated the influences of maternal distress in pregnancy on birth outcomes and subsequent child development [16, 17], prospective population-based human studies of the effect of maternal prenatal distress on fetal growth trajectories in pregnancy - to the best of our knowledge - do not exist. Birth outcomes, such as birth weight, are only crude summary measures of intrauterine growth at the end of pregnancy and cannot provide information on fetal growth characteristics of the fetal head, abdomen, femur and body across different time periods in pregnancy. Furthermore, individuals can reach the same birth weight by different fetal growth trajectories [18]. Therefore, in the current population-based cohort study, we examined the effect of maternal prenatal distress not only on birth weight but also on repeatedly measured fetal growth parameters such as head and abdominal circumference and femur length in mid- and late pregnancy. We hypothesized that maternal distress in pregnancy negatively affects fetal size and growth from mid-pregnancy until birth.

Methods

Design

The current study was embedded in the Generation R Study, a population-based multi-ethnic cohort from fetal life until young adulthood in Rotterdam, The Netherlands. The Generation R Study has previously been described in detail [19, 20]. The cohort includes 9,778 mothers and their children that were born between April 2002 and January 2006. Assessments in pregnant women consisted of physical examinations, fetal ultrasounds, biological samples and questionnaires. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam (number: MEC 198.782/2001/31). Written informed consent was obtained from all pregnant women.

Population for analysis

Of the total cohort of 9,778 mothers, 8,880 (91%) were enrolled in pregnancy [20]. From the current study 104 fetal deaths and 93 mothers with twin pregnancies were excluded because growth potentials for individual fetuses in multiple pregnancies are not comparable to singleton pregnancies. Furthermore, for mothers with multiple pregnancies, data on their second (n = 500) or third (n = 8) pregnancy during the study were also excluded in order to avoid effects of paired data in our data-analysis. The remaining 8,130 mothers were eligible. There were 45 losses to follow-up during pregnancy. In 22.2% (n = 1806) of the eligible mothers no information on any of the three types of maternal distress was available. In addition, for 11 mothers there was no data on fetal ultrasound measurements. Of the remaining 6,313 (77.7% of 8,130) mothers, most mothers (n = 5,976, 94.7%) had two ultrasound assessments in mid- and late pregnancy, 337 (5.3%) mothers attended only one ultrasound assessment.

Maternal psychological distress in pregnancy

Information on maternal distress was obtained by postal questionnaires at 20 weeks of gestation. Maternal symptoms of depression and anxiety in pregnancy were assessed with the Brief Symptom Inventory (BSI), a validated self-report questionnaire consisting of 53 items [21, 22]. These items define a spectrum of psychiatric symptoms in the preceding seven days. For the current study, the 6-item anxiety scale, e.g. "nervousness or shaking inside", and the 6-item depression scale, e.g. "feeling lonely" were used. Each item was rated on 5-point uni-dimensional rating scales ranging from '0' (not at all) to '4' (extremely). The total scores for symptoms of depression and anxiety were calculated by first summing the item scores (range: 0-4) and then dividing by the number of endorsed items. The internal consistencies in this study were $\alpha = 0.80$ for the depression scale and $\alpha = 0.75$ for the anxiety scale. Those mothers who scored in the top 15 percent were considered to be highly anxious or depressive in line with earlier studies using a very similar cut-off when defining increased antenatal anxiety [17].

Family stress was assessed by the 7th subscale General Functioning (GF) of the Family Assessment Device (FAD) [23]. GF is a validated overall self-report measure of health or pathology of the family, which consists of 12 items. The item scores were summed and then divided by 12 yielding a total score from 1 to 4. If the GF score is higher than 2.17 (cutting point), then family functioning is considered to be unhealthy. In the current study, just as in the Ontario Child Health Study, 10 percent of the families scored above this cutting point [23]. The internal consistency of GF was $\alpha = 0.90$.

Fetal ultrasound measurements and birth weight

Trained sonographers conducted fetal ultrasound examinations at the visits to the research centers in early (gestational age < 18 weeks), mid-pregnancy (gestational age 18-25 weeks) and late pregnancy (gestational age ≥ 25 weeks). These ultrasound examinations were used for establishing gestational age and assessing fetal growth characteristics.

In the current study, online measurements included head and abdominal circumference, and femur length in mid- and late pregnancy that were all measured to the nearest millimeter using standardized techniques. Estimated fetal weight was calculated using the formula by Hadlock including head and abdominal circumference, and femur length [24]. An accurate estimation of fetal weight cannot be achieved before 18 weeks of gestation [24]. Birth weight was obtained from medical records completed by midwives and gynecologists.

Covariates

Information on maternal age, pre-pregnancy body mass index, educational level, ethnicity and parity (0, or ≥ 1) was obtained by questionnaire at enrolment. Education was divided in five categories: Ranging from 'primary education only' to 'higher education with a university degree'. Ethnicity of the mother was based on the country of birth of herself and her parents. Maternal height was measured during the first visit to the research center. Maternal use of alcohol during pregnancy ('no alcohol use' / 'alcohol use until pregnancy was known' / 'continuing to drink alcohol when pregnancy was known') and smoking (classified analogous to alcohol use) were asked for at inclusion and 20 and 30 weeks of pregnancy. Fetal gender and information on gestational diabetes, preeclampsia and maternal hypertension during pregnancy were obtained from medical records.

Statistical analysis

Multiple linear regression was used to examine the associations of maternal psychological distress with fetal size in mid- and late pregnancy and birth weight. All models were controlled for gestational age, maternal education, known determinants of fetal development, i.e. maternal height, age, body mass index, ethnicity, smoking during pregnancy, parity, gestational diabetes, preeclampsia, hypertension and fetal gender [25]. In addition, all models were adjusted for maternal anxious symptoms in pregnancy or for family stress. To avoid collinearity and over-adjustment maternal anxious and depressive symptoms in pregnancy were not included in the same model. Anxiety and depression as measured by the BSI were highly comorbid (correlation: $r = 0.7$, $p < .001$). Maternal alcohol use during pregnancy did not improve the model fit of the different models and was therefore not included in the analysis. Continuous measures of maternal distress were used in our main analyses and were

expressed in standard deviations. Although the distributions of the total scores of the measurements of maternal distress were skewed, the residuals of the used models were roughly normally distributed, and thus the scores were not transformed.

The associations of the three different types of maternal distress with repeatedly measured parameters of fetal growth were analyzed using longitudinal multilevel analysis to account for the dependency between measurements in the same subject [26]. The best fitting model was built using fractional polynomials of gestational age [27]. Then, the respective type of maternal distress was brought into the model as main determinant. The interaction term of maternal distress with gestational age was included in the model to determine whether affective symptoms and family stress affected the slope of the curves. The following models were used:

Head circumference = β_0 + [β_1*type of maternal distress] + [β_2*gestational age + β_3*gestational age^2] + [β_4*gestational age^2*ln(gestational age)] + [β_5*type of maternal distress*gestational age].

Femur length = β_0 + [β_1*type of maternal distress] + [β_2*gestational age] + [β_3*gestational age^3] + [β_4*type of maternal distress*gestational age].

Fetal weight gain = β_0 + [β_1*type of maternal distress] + [β_2*gestational age] + [β_3*gestational age*ln(gestational age)] + [β_4*type of maternal distress*gestational age].

The model of abdominal circumference was the same as the model of head circumference. Models were based on 11,856 observations for head circumference, 11,915 observations for abdominal circumference, 11,925 observations for femur length and 18,010 observations for fetal and birth weight. All models were controlled for potential confounders. In a second step, all models were additionally adjusted for maternal anxious symptoms in pregnancy or for family stress as well as for the respective interaction with gestational age to determine whether a type of maternal distress was independently related to fetal growth trajectories. The betas of the maternal distress score (per standard deviation) represent the difference in the slope of the fetal growth-curve in mm per week. We reran all analyses, using dichotomized measures of distress as main determinants, to illustrate the results. SPSS for Windows (version 11.0) and SAS v.8.2 (Stata Corporation, College Station, TX, USA), including the Proc Mixed module for longitudinal multilevel analysis, were used for data analysis.

Results

Table 1 presents the subject characteristics of the current study. Family stress in pregnancy was significantly and moderately correlated with both anxiety ($r = 0.3$, $p < .001$) and depression ($r = 0.4$, $p < .001$). Head and abdominal circumference and femur length were all strongly and significantly correlated with each other ($r = 0.6$, $p < .001$) in late pregnancy. Correlations between theses ultrasound measurements in mid-pregnancy were similar (data not shown).

Table 1. Maternal and child characteristics (n = 6,313)

Maternal characteristics	Mean (SD)*
Age, y	29.8. (5.2)
Height, cm	167.5 (7.4)
Pre-pregnancy BMI, kg/m^2	23.6 (4.3)
Parity (% nulli)	61.5
Education (%)	
Primary education	9.6
Secondary education 1st phase	15.4
Secondary education 2nd phase	31.0
Higher education 1st phase	20.4
Higher education 2nd phase	23.6
Ethnicity (%)	
Dutch	52.6
Cape Verdian	3.8
Moroccan	5.6
Dutch Antilles	3.4
Surinamese	8.8
Turkish	8.3
Other Western	12.0
Other non-western	5.6
Smoking during pregnancy (%)	
No	74.9
Until pregnancy was known	7.5
Continued during pregnancy	17.6
Alcohol use in pregnancy (%)	
No	45.1
Until pregnancy was known	12.9
Continued during pregnancy	42.0
Gestational diabetes (%, Yes)	1.1
Preeclampsia (%, Yes)	1.8
Hypertension (%, Yes)	4.1
Anxiety during pregnancy	0.31 (0.5)
Depression during pregnancy	0.26 (0.5)
Family functioning during pregnancy	1.58 (0.5)
Child characteristics	**Mean (SD)***
Gender (% girls)	50.2
Gestational age in early pregnancy, weeks, median (95% range)	13.2 (10.6 – 17.5)
Gestational age in mid-pregnancy, weeks, median (95% range)	20.5 (18.6 – 23.3)
Head circumference in mid-pregnancy (mm)	179.4 (14.4)
Abdominal circumference in mid-pregnancy (mm)	156.9 (14.7)
Femur length in mid-pregnancy (mm)	33.5 (3.55)
Estimated fetal weight in mid-pregnancy (grams)	381.2 (92.7)
Gestational age in late pregnancy, weeks, median (95% range)	30.3 (28.4 – 32.9)
Head circumference in late pregnancy (mm)	284.8 (12.3)
Abdominal circumference in late pregnancy (mm)	263.8 (16.4)
Femur length in late pregnancy (mm)	57.4 (3.0)
Estimated fetal weight in late pregnancy (grams)	1615 (254)
Birth weight, grams	3,417 (556)
Gestational age at birth, weeks	39.9 (1.8)

* Unless otherwise indicated

Table 2. Associations of measures of maternal distress during pregnancy with fetal size in mid- and late pregnancy and size at birth[a]

	Estimated fetal weight in mid-pregnancy (grams)	Estimated fetal weight in late pregnancy (grams)	Birth weight (grams)
Type of maternal distress	B[b] (95% CI[c])	B[b] (95% CI[c])	B[b] (95% CI[c])
Depressive symptoms, per SD	-0.11 (-1.36; 1.14)	-0.68 (-5.96; 4.59)	0.11 (-11.52; 11.74)
Anxious symptoms, per SD	-0.05 (-1.30; 1.20)	-2.13 (-7.22; 2.97)	-13.02 (-24.59; -1,45)*
Family stress, per SD	0.11 (-1.19; 1.41)	-0.58 (-5.70; 4.54)	-10.27 (-22.27; 1.71)

[a]Adjusted for gestational age in mid- or late pregnancy or at birth, fetal gender, maternal age, height, body mass index, education, ethnicity, smoking during pregnancy, parity, gestational diabetes, hypertension in pregnancy, preeclampsia and for maternal anxious symptoms in pregnancy in case of family stress or for family stress in case of maternal anxious/ depressive symptoms

[b] Betas represent the in- or decrease of the fetal size characteristics in grams per standard deviation of the continuous measures of forms of maternal psychological distress in pregnancy

[c] CI: confidence interval

* $p \leq .05$

A non-response analysis showed that, in comparison to non-responders, mothers included in the study were more likely to be Dutch (52.6% vs. 30.6%, $\chi^2 = 327.93$, df = 7, $p < .001$) and to be higher educated (% higher education with an university degree 23.7 % vs. 13.3%, $\chi^2 = 266.32$, df = 4, $p < .001$). Children of mothers in the study had a higher birth weight (mean, 3.416 grams (SD, 556) vs. 3.343 grams (SD, 581), t = 4.83, $p < .001$) and gestational age at birth (mean, 39.9 (SD, 1.8) vs. 39.6 (SD, 2.3), t = 5.99, $p < .001$) than children of non-responders.

Table 2 shows that maternal distress was not related to estimated fetal weight in mid-pregnancy. In contrast, a crude analysis demonstrated that all types of maternal distress were negatively associated with fetal weight in late pregnancy. However, these relationships disappeared after controlling for potential confounders (Table 2). Very similarly, maternal distress was negatively related to birth weight before adjustments were made (data not shown). But after controlling for potential confounders, only anxiety symptoms were associated with lower birth weight. A 1 standard deviation increase in maternal anxious symptoms resulted in a 13.02 grams (95% CI: -24.59; -1,45, p = .027) lower birth weight (Table 2).

Table 3 presents the adjusted associations between continuous measures of maternal distress and fetal growth characteristics. Maternal distress - affective symptoms as well as family stress - had a negative impact on the growth trajectories of all fetal body parts and fetal weight gain. There were only three exceptions, maternal depressive and anxious symptoms were not related to growth of the fetal femur and family stress was not linked to growth patterns of the fetal abdomen. Next, we additionally adjusted these associations for family stress or maternal anxious symptoms. The associations of affective symptoms as well as family stress with fetal weight gain were attenuated but all remained statistically significant. For example, a 1 standard deviation increase of depressive symptoms reduced fetal weight gain by 1.18 grams (95% CI: -1.81; -0.54, $p < .001$) per week (other data not shown). Similarly, we adjusted the relation between distress and specific fetal body parts. Maternal anxious symptoms were still related to fetal growth trajectories of the fetal head and abdomen and

Table 3. Associations of maternal depressive and anxious symptoms and family stress in pregnancy with fetal growth[a]

	Difference in head circumference growth (mm/week)
Type of maternal distress	B[b] (95% CI[c]) *P* value
Depressive symptoms, per SD	-0.033 (-0.055; -0.011) .007
Anxious symptoms, per SD	-0.032 (-0.054; -0.009) .004
Family stress, per SD	-0.024 (-0.049; -0.019) .04
	Difference in abdominal circumference growth (mm/week)
Type of maternal distress	B[b] (95% CI[c]) *P* value
Depressive symptoms, per SD	-0.040 (-0.071; -0.009) .01
Anxious symptoms, per SD	-0.043 (-0.074; -0.012) .007
Family stress, per SD	-0.030 (-0.062; 0.001) .06
	Difference in femur length growth (mm/week)
Type of maternal distress	B[b] (95% CI[c]) *P* value
Depressive symptoms, per SD	-0.004 (-0.010; 0.001) .12
Anxious symptoms, per SD	-0.003 (-0.009; 0.002) .23
Family stress, per SD	-0.007 (-0.012; -0.002) .01
	Difference in fetal weight gain (grams/week) until birth
Type of maternal distress	B[b] (95% CI[c]) *P* value
Depressive symptoms, per SD	-1.36 (-1,93; -0,79) <.001
Anxious symptoms, per SD	-1.23 (-1,80; -0,66) <.001
Family stress, per SD	-1.10 (-1.67; -0.53) <.001

[a] Adjusted for fetal gender, maternal age, height, body mass index, education, ethnicity, smoking during pregnancy, parity, gestational diabetes, hypertension in pregnancy and preeclampsia

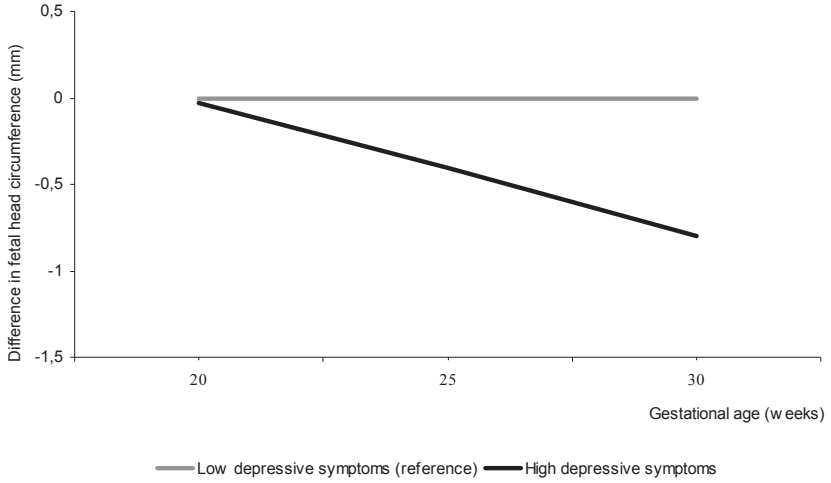
[b] Betas represent the difference in the slope of the fetal growth-curve expressed in mm (in grams for fetal weight gain) per week per standard deviation of the continuous measures of forms of maternal psychological distress in pregnancy

[c] CI: confidence interval

depressive symptoms were still linked to a reduced growth of the fetal head. A 1 standard deviation increase of anxious symptoms resulted in a growth of fetal head circumference that was 0.030 mm (95% CI: -0.054; -0.005, p = .017) lower per week and reduced the growth of the fetal abdomen by 0.040 mm (95% CI: -0.074; -0.006, p = .020) (other data not shown). All other associations were no longer significant (data not shown).

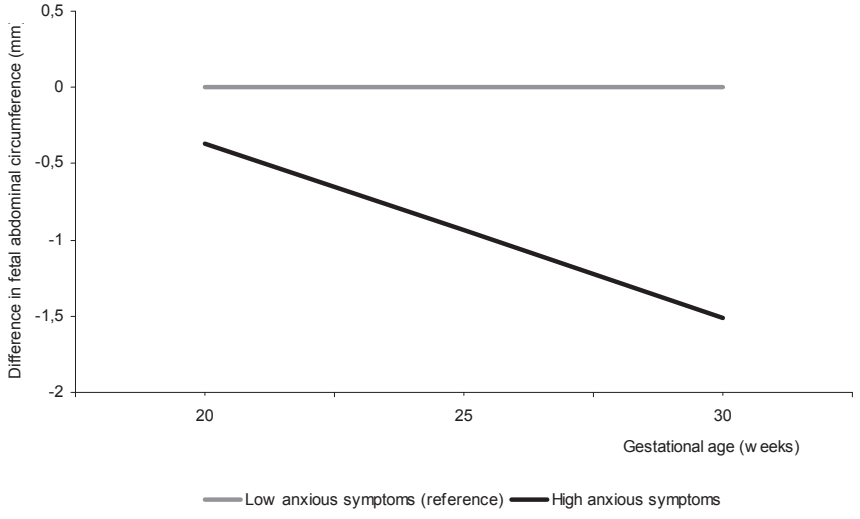
Figure 1 and 2 illustrate associations between maternal distress and fetal growth parameters. Figure 1 shows the estimated difference in fetal growth of head circumference between mothers who reported low depressive symptoms during pregnancy compared to mothers who reported high depressive symptoms. This figure shows differences instead of main effects of depressive symptoms on fetal head circumference as the effect sizes were small (max. 0.8 mm at 30 weeks of gestation) and the growth rate was comparatively large (from 172 mm at 20 weeks of gestation to 282 mm at 30 weeks of gestation). Model estimates were most stable between 20 and 30 weeks of gestation and this period represents 90% of the data on head growth. Total effect size of high depressive symptoms on fetal head circumference varied from 0.02% (at 20 weeks of gestational age) to 0.28% (at 30 weeks of gestational age). Figure 2 shows the estimated difference in fetal growth of abdominal circumference between mothers who reported low anxious symptoms and mothers who reported high anxious

Figure 1. Differences in growth of fetal head circumference between pregnant women with high and low depressive symptoms during pregnancy



Mothers who scored in the top 15 percent of the depression symptoms were considered to be highly depressive during pregnancy. Values represent statistically significant estimated differences (p = .032) in growth of fetal head circumference between the two groups of mothers based on the linear mixed models, adjusted for fetal gender, maternal age, height, body mass index, education, ethnicity, smoking during pregnancy, parity, gestational diabetes, pre-eclampsia, maternal hypertension during pregnancy and family functioning in pregnancy and its interaction with gestational age.

Figure 2. Differences in fetal abdominal circumference between mothers with high and low anxious symptoms during pregnancy



Mothers who scored in the top 15 percent of the anxiety symptoms were considered to be highly anxious during pregnancy. Values represent statistically significant estimated differences (p = .018) in fetal abdominal circumference between the two groups of mothers based on the linear mixed models, adjusted for fetal gender, maternal age, height, body mass index, education, ethnicity, smoking during pregnancy, parity, gestational diabetes, pre-eclampsia, maternal hypertension during pregnancy and family functioning in pregnancy and its interaction with gestational age.

symptoms. A high level of maternal anxious symptoms reduced fetal abdominal circumference by 0.25% at 20 weeks of gestation and by 0.58% at 30 weeks of gestation. Overall, the remaining mixed model analyses with dichotomous main determinants showed very similar results as the analyses with continuous measures of maternal psychological distress (remaining data not shown).

Discussion

In the current study, we showed that affective symptoms during pregnancy led to a reduced growth of, in particular, fetal head and abdominal circumference. Different forms of maternal distress were negatively related to fetal weight gain during pregnancy, but only maternal anxious symptoms during pregnancy led to a lower birth weight.

In line with earlier research linking maternal psychological distress in pregnancy to lower birth weight [9, 12-14] and a smaller head size at birth [9] our study showed a negative association between maternal anxious symptoms and birth weight. But birth outcome measures, such as birth weight, are only summative measures at the endpoint of a long, rapid and non-linear period of intrauterine growth. Moreover, birth weight does not provide information on the trajectories of fetal growth. This is of particular importance because the same birth size can be obtained by different fetal growth patterns [18]. While undergoing fetal growth restriction due to environmental influences an individual fetus may still reach a normal birth weight because of his high genetic growth potential. Nevertheless the fetal growth restriction may result in changes of fetal physiology and lifetime health [28].

In contrast to our finding that maternal distress did not predict fetal size in mid- and late pregnancy, a single cross-sectional study reported an association between maternal psychological distress and fetal size, indexed by fetal weight [29]. This study was based on a small sample (n = 98) with measurements in mid-pregnancy only and an incomplete control for confounders.

In our study, maternal distress was related to size at birth but not to fetal size in mid- and late pregnancy, which suggests that influences of maternal distress on fetal growth are strongest in the last trimester of pregnancy. This is not surprising because the growth rate is highest in that period of pregnancy. The finding may also reflect that effects of maternal distress on fetal growth are cumulative and easier to detect in the last trimester of pregnancy because of an increasing discriminative power of the used measures.

The results of the current study showed that maternal distress was more consistently associated with reduced fetal weight gain, and growth of the fetal head and abdomen in comparison to growth patterns of the fetal femur. Probably, maternal distress during pregnancy affects fetal organ development more than the development of fetal distal body parts and bone structure.

Our findings support the notion that maternal distress affects fetal head growth. As head circumference is known to correlate with brain volume [30], repeated measures of fetal head growth can be interpreted as indicators of fetal brain development. Earlier studies reported a relation of maternal psychological distress in pregnancy with child outcomes, such as behavioral problems or poorer growth in infancy [16, 17]. Moreover, previous research showed that intrauterine growth restriction indexed by birth length is associated with childhood behavioral problems and that head circumference at birth predicts cognitive functioning in childhood [31, 32]. Possibly fetal head growth is an intermediate in the relation of maternal psychological distress and subsequent child development.

Several mechanisms have been put forward that may explain the association between maternal distress in pregnancy and fetal growth. Human and non-human research suggests that maternal stress and distress during pregnancy leads to an elevated maternal HPA-axis activity, which causes an increased release of glucocorticoids [7, 10, 29, 33, 34], that in turn negatively affect fetal development [7, 10, 29]. Maternal stress hormones may be transduced to the fetus by transplacental transport and by stress-induced release of placental hormones that enter the fetal circulation [34]. A study by Gitau et al. (1998) showed that maternal cortisol levels are strongly correlated with fetal levels, although fetal concentrations are lower compared to maternal concentrations [35]. Glucocorticoids are involved in fetal tissue proliferation and differentiation and are growth inhibitory [34, 36-38]. In addition, their concentrations are elevated in response to prenatal environmental events, such as maternal undernutrition, placental insufficiency and restriction of placental blood flow [39].

Our results might also be explained by an underlying common genetic factor that affects both maternal levels of psychological distress and fetal growth trajectories. Although we controlled for genetic effects on fetal growth by adjusting for maternal height and pre-pregnancy body-mass index, residual genetic influences are likely.

The main strength of this large prospective population-based cohort study was that the repeated fetal ultrasound assessments were combined with information on birth weight, so that we were able to assess fetal growth across the entire range from mid-pregnancy until birth. In addition, we could control for a large number of potential confounders known to affect fetal development.

Several potential limitations of the current study must be considered. As the maternal psychological distress was only assessed at 20 weeks pregnancy, we do not know whether affective symptoms and family stress reported by mothers varied in intensity or were persistent throughout pregnancy. Secondly, the anxiety scale and the depression scale of the BSI were strongly correlated. It seems plausible that these scales measure very similar concepts. This reflects the comorbidity between anxiety and depression, which has frequently been reported by previous studies [40]. We were not able to disentangle whether maternal anxiety and depression during pregnancy have independent effects on fetal growth trajectories, because of collinearity and possible over-adjustment in our analysis. In addition, our data

do not allow us to determine, which physiological mechanisms, e.g. HPA-axis activity level, may account for the findings of the current study. Finally, as data on maternal distress were more complete in Dutch and higher-educated mothers whose children had a higher birth weight and gestational age, we cannot rule out non-random selection effects on fetal growth trajectories.

In conclusion, maternal psychological distress during pregnancy affects fetal development. Future research should address mechanisms underlying the relation between maternal psychological distress and fetal growth, e.g. dysregulation of the HPA-axis, and their long-term effects on child development. Furthermore, our findings highlight the importance of factors resulting in distress in pregnant women because they may affect the fetus. Information about distress can easily be obtained by means of maternal questionnaires. Pregnant women at elevated risk could then be invited to participate, for example, in stress reduction programs.

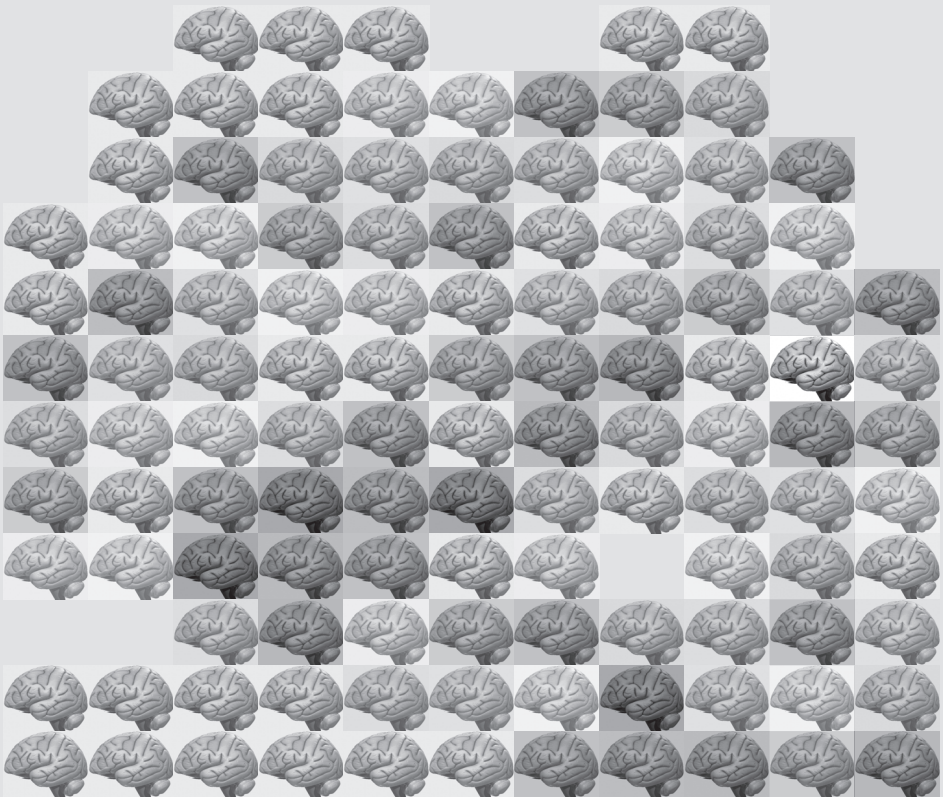
References

1. Ferreira AJ. Emotional factors in prenatal environment. A review. *J Nerv Ment Dis.* Jul 1965;141(1):108-118.
2. Herrenkohl LR. Prenatal stress reduces fertility and fecundity in female offspring. *Science.* Nov 30 1979;206(4422):1097-1099.
3. Lesage J, Del-Favero F, Leonhardt M, et al. Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behaviour disturbances in the aged rat. *J Endocrinol.* May 2004;181(2):291-296.
4. Pinto ML, Shetty PS. Influence of exercise-induced maternal stress on fetal outcome in Wistar rats: inter-generational effects. *Br J Nutr.* May 1995;73(5):645-653.
5. Nakano Y, Oshima M, Sugiura-Ogasawara M, Aoki K, Kitamura T, Furukawa TA. Psychosocial predictors of successful delivery after unexplained recurrent spontaneous abortions: a cohort study. *Acta Psychiatr Scand.* Jun 2004;109(6):440-446.
6. Kurki T, Hiilesmaa V, Raitasalo R, Mattila H, Ylikorkala O. Depression and anxiety in early pregnancy and risk for preeclampsia . *Obstet Gynecol.* Apr 2000;95(4):487-490.
7. Field T, Diego M, Dieter J, et al. Prenatal depression effects on the fetus and the newborn. *Infant Behavior & Development.* MAY 2004;27(2):216-229.
8. Hedegaard M, Henriksen TB, Sabroe S, Secher NJ. Psychological distress in pregnancy and preterm delivery. *Bmj.* Jul 24 1993;307(6898):234-239.
9. Lou HC, Hansen D, Nordentoft M, et al. Prenatal stressors of human life affect fetal brain development. *Dev Med Child Neurol.* Sep 1994;36(9):826-832.
10. Mancuso RA, Schetter CD, Rini CM, Roesch SC, Hobel CJ. Maternal prenatal anxiety and corticotropin-releasing hormone associated with timing of delivery. *Psychosomatic Medicine.* SEP-OCT 2004;66(5):762-769.
11. Orr ST, James SA, Blackmore Prince C. Maternal prenatal depressive symptoms and spontaneous preterm births among African-American women in Baltimore, Maryland. *Am J Epidemiol.* Nov 1 2002;156(9):797-802.
12. Paarlberg KM, Vingerhoets AJJM, Passchier J, Dekker GA, Heinen AGJJ, van Geijn HP. Psychosocial predictors of low birthweight: a prospective study. *British Journal of Obstetrics and Gynaecology.* AUG 1999;106(8):834-841.
13. Rondo PH, Ferreira RF, Nogueira F, Ribeiro MC, Lobert H, Artes R. Maternal psychological stress and distress as predictors of low birth weight, prematurity and intrauterine growth retardation. *Eur J Clin Nutr.* Feb 2003;57(2):266-272.
14. Wadhwa PD, Sandman CA, Porto M, Dunkel-Schetter C, Garite TJ. The association between prenatal stress and infant birth weight and gestational age at birth: a prospective investigation. *Am J Obstet Gynecol.* Oct 1993;169(4):858-865.
15. Obel C, Hedegaard M, Henriksen TB, Secher NJ, Olsen J. Stressful life events in pregnancy and head circumference at birth. *Dev Med Child Neurol.* Dec 2003;45(12):802-806.
16. Rahman A, Iqbal Z, Bunn J, Lovel H, Harrington R. Impact of maternal depression on infant nutritional status and illness: a cohort study. *Arch Gen Psychiatry.* Sep 2004;61(9):946-952.
17. O'Connor TG, Heron J, Glover V. Antenatal anxiety predicts child behavioral/emotional problems independently of postnatal depression. *J Am Acad Child Adolesc Psychiatry.* Dec 2002;41(12):1470-1477.
18. Bloomfield FH, Oliver MH, Harding JE. The late effects of fetal growth patterns. *Arch Dis Child Fetal Neonatal Ed.* Jul 2006;91(4):F299-304.
19. Hofman A, Jaddoe VW, Mackenbach JP, et al. Growth, development and health from early fetal life until young adulthood: the Generation R Study. *Paediatr Perinat Epidemiol.* Jan 2004;18(1):61-72.
20. Jaddoe VW, Mackenbach JP, Moll HA, et al. The Generation R Study: Design and cohort profile. *Eur J Epidemiol.* Jul 7 2006.

21. Derogatis LR. *Brief Symptom Inventory (BSI): Administration, scoring and procedures. Manual, third edition*. Minneapolis, MN; 1993.
22. Beurs d. *Brief Symptom Inventory, handleiding*. Leiden, The Netherlands; 2004.
23. Byles J, Byrne C, Boyle MH, Offord DR. Ontario-Child-Health-Study - Reliability and Validity of the General Functioning Subscale of the Mcmaster Family Assessment Device. *Family Process.* MAR 1988;27(1):97-104.
24. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. *Radiology.* Feb 1984;150(2):535-540.
25. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. *Bull World Health Organ.* 1987;65(5):663-737.
26. Twisk JWR. *Applied Multilevel Analysis. A practical Guide*. Cambridge, UK: University Press; 2006.
27. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. *Int J Epidemiol.* Oct 1999;28(5):964-974.
28. Hanson M. Birth weight and the fetal origins of adult disease. *Pediatr Res.* Oct 2002;52(4):473-474.
29. Diego MA, Jones NA, Field T, et al. Maternal psychological distress, prenatal cortisol, and fetal weight. *Psychosom Med.* Sep-Oct 2006;68(5):747-753.
30. Cooke RW, Lucas A, Yudkin PL, Pryse-Davies J. Head circumference as an index of brain weight in the fetus and newborn. *Early Hum Dev.* Oct 1977;1(2):145-149.
31. Wiles NJ, Peters TJ, Heron J, Gunnell D, Emond A, Lewis G. Fetal growth and childhood behavioral problems: results from the ALSPAC cohort. *Am J Epidemiol.* May 1 2006;163(9):829-837.
32. Gale CR, O'Callaghan FJ, Bredow M, Martyn CN, Avon Longitudinal Study of Parents and Children Study T. The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. *Pediatrics.* Oct 2006;118(4):1486-1492.
33. Barbazanges A, Piazza PV, Le Moal M, Maccari S. Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *J Neurosci.* Jun 15 1996;16(12):3943-3949.
34. Huizink AC, Mulder EJ, Buitelaar JK. Prenatal stress and risk for psychopathology: specific effects or induction of general susceptibility? *Psychol Bull.* Jan 2004;130(1):115-142.
35. Gitau R, Cameron A, Fisk NM, Glover V. Fetal exposure to maternal cortisol. *Lancet.* Aug 29 1998;352(9129):707-708.
36. Fowden AL, Li J, Forhead AJ. Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? *Proc Nutr Soc.* Feb 1998;57(1):113-122.
37. Fowden AL, Forhead AJ. Endocrine mechanisms of intrauterine programming. *Reproduction.* May 2004;127(5):515-526.
38. Reinisch JM, Simon NG, Karow WG, Gandelman R. Prenatal exposure to prednisone in humans and animals retards intrauterine growth. *Science.* Oct 27 1978;202(4366):436-438.
39. Challis JR, Sloboda D, Matthews SG, et al. The fetal placental hypothalamic-pituitary-adrenal (HPA) axis, parturition and post natal health. *Mol Cell Endocrinol.* Dec 20 2001;185(1-2):135-144.
40. Beekman AT, de Beurs E, van Balkom AJ, Deeg DJ, van Dyck R, van Tilburg W. Anxiety and depression in later life: Co-occurrence and communality of risk factors. *Am J Psychiatry.* Jan 2000;157(1):89-95.

2.3

Maternal smoking during pregnancy and child behavior problems



Abstract

Background: Several studies showed that maternal smoking in pregnancy is related to behavioral and emotional disorders in the offspring. It is unclear whether this is a causal association, or can be explained by other smoking-related vulnerability factors for child behavioral problems.

Methods: Within a population-based birth cohort, both mothers and fathers reported on their smoking habits at several time-points during pregnancy. Behavioral problems were measured with the Child Behavior Checklist in 4680 children at the age of 18 months.

Results: With adjustment for age and gender only, children of mothers who continued smoking during pregnancy had higher risk of Total Problems (OR 1.59, 95% confidence interval: 1.21 – 2.08) and Externalizing problems (OR 1.45, 95% confidence interval: 1.15 – 1.84), compared to children of mothers who never smoked. Smoking by father when mother did not smoke, was also related to a higher risk of behavioral problems. The statistical association of parental smoking with behavioral problems was strongly confounded by parental characteristics, chiefly socioeconomic status and parental psychopathology; adjustment for these factors accounted entirely for the effect of both maternal and paternal smoking on child behavioral problems.

Conclusions: Maternal smoking during pregnancy, as well as paternal smoking, occurs in the context of other factors that place the child at increased developmental risk, but may not be causally related to the child's behavior. It is essential to include sufficient information on parental psychiatric symptoms in studies exploring the association between prenatal cigarette smoke exposure and behavioral disorders.

Introduction

Maternal smoking during pregnancy leads to intrauterine growth restriction, and to more perinatal morbidity and mortality [1, 2]. The neurodevelopmental consequences of prenatal nicotine exposure are, despite a large body of research, less clear. Products of cigarette smoke, like carbon monoxide, tar, and nicotine, have been proposed to directly affect the fetal brain [3] indirect effects on the brain include fetal hypoxia due to vasoconstriction in the placenta and maternal and fetal undernutrition [1, 4]. An alternative explanation for the association of prenatal maternal smoking with neurodevelopment in the offspring centers around the epiphenomenona of smoking, such as parental psychopathology, low socioeconomic status, coabuse of other substances, and poor prenatal care [5].

Several methodological problems limit the interpretation of the association between maternal smoking during pregnancy and offspring behavioral disorders. First, many researchers relied on retrospective assessment of prenatal smoking, which may induce recall bias. Moreover, retrospective assessment may hamper the identification of and vulnerability periods during gestation. Second, differences in measurement of potential confounding variables, such as demographic variables, socioeconomic factors, other maternal substance use, and familial psychopathology, led to inconclusive findings [5-7]. Some studies reported significant attenuation, some reported complete erosion, whereas others reported no effect of these confounding variables on the association between smoking during pregnancy and offspring behavior.

In this study, we examine the hypothesis that maternal smoking during pregnancy is related to behavioral problems, using data from the general population with assessment of smoking exposure repeatedly during pregnancy. We address the following questions: 1) Is there an effect of maternal smoking during pregnancy on the child behavioral problems at the age of 18 months, that cannot be explained by parental characteristics such as parental educational level, family income, and parental psychiatric symptoms? and 2) Is there a dose-response effect of prenatal active and passive smoking on children's behavioral problems?

Materials and methods

Setting

This study was conducted within the Generation R Study, a population-based cohort in Rotterdam, the Netherlands [8]. Enrolment was aimed in early pregnancy. All children were born between April 2002 and January 2006 and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. The study has been approved by the Medical

Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all adult participants.

Study population

In total, 7654 prenatally included live born children and their mothers were approached for postnatal consent. Children without information on maternal smoking habits in pregnancy were excluded (n=348, 4.5%). Thirty-four children deceased in the first few months after birth. The remaining 7272 children were eligible for the present study. Mothers of 877 children did not give full consent for postnatal participation. Another 1715 mothers did not complete the 18-month-questionnaire. Information on child behavioral problems at age 18 months was available in 4680 toddlers (64.4% of 7272). Some mothers participated with two children (n=341), or three children (n=5). Since results did not differ after random exclusion of one or two of these siblings, they were included in the analyses.

Maternal smoking during pregnancy

Information about maternal smoking was obtained by questionnaires in the first, second, and third trimester. At enrolment, we asked whether mother smoked before pregnancy and during the first three months of pregnancy. In the second and third questionnaire, mothers were asked whether they smoked in the past 3 months. Maternal smoking was categorized on the basis of all three questionnaires into 'never smoked', 'quit smoking before pregnancy', 'quit smoking in first trimester', and 'continued smoking'. In smokers, the number of cigarettes smoked daily was categorized, according to the highest amount reported. Both mothers and fathers were asked on paternal smoking behavior. We used maternal information on paternal smoking when fathers did not complete this question (n=1104). Furthermore, mothers were asked whether they were exposed to environmental smoke at home in the second and third trimester. We categorized parental active and passive smoking as 'no active or passive smoking', 'father smoked outside, mother did not smoke', 'father smoked indoors, mother did not smoke', and 'mother smoked'.

Child behavioral and emotional problems

The Child Behavior Checklist for toddlers (CBCL/1½-5) was used to obtain standardized parent reports of children's problem behaviors. The Total Problems score is the sum score of the 99 problem items. The broadband scale Internalizing is the sum score of items in four syndrome scales: Emotionally Reactive, Anxious/Depressed, Somatic Complaints, and Withdrawn. Externalizing is the sum score of Attention Problems and Aggressive Behavior. Each item is scored 0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true,

based on the preceding two months. Good reliability and validity have been reported for the CBCL [9]. We used the borderline cut-off score (83rd percentile of a Dutch norm group [10]) to classify children as having behavioral problems in the borderline / clinical range.

Covariates

Gestational age at birth, birth weight and gender of the infant were obtained from midwife and hospital registries at birth. Parental age was assessed at enrolment. We classified marital status of the pregnant woman into married / cohabiting vs. single. Educational level of the mother and the father was assessed by the highest completed education. The child was of non-Dutch origin if one of the parents was born abroad. If both parents were born abroad, the country of birth of the mother decided on the ethnic background. We classified national origin into four categories (a: Dutch or other Western, b: Turkish or Moroccan, c: Surinamese or Antillean, or d: other non-Western). Family income was categorized into < 1200 euro, 1200 – 2000 euro or > 2000 euro net a month. To assess maternal and paternal psychopathology in mid-pregnancy, we used the Brief Symptom Inventory (BSI) [11,12]. The score on the Global Severity Index was standardized. In mid-pregnancy, we asked both parents whether they had a delinquent past.

Statistical analyses

Differences in baseline characteristics between children with and without behavioral problems were compared with the chi-square statistic, independent t-tests, and Mann-Whitney U tests. We used the chi-square and the Kruskal-Wallis test for comparison of parental characteristics between the different maternal smoking categories. Successive logistic regression models with introduction of a new set of variables are presented to show whether the association between maternal smoking and behavioral problems holds-up when potential confounders are controlled. Interaction terms of maternal smoking with all significant confounding variables were tested and included at $\alpha = 0.15$. In addition, we analyzed the dose-response relationships of active and of passive smoking with child behavioral problems. Measures of association are presented with their 95% confidence intervals (CI). Statistical analyses were carried out with the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

Response analyses

Analyses of missing data showed that children without information on behavior had on average 113 (95% confidence interval 85 – 140, t=8.0, p<0.001) grams lower birth weight, had shorter gestation (median 39.7 (95% range 34.7 – 42.3) weeks vs. 40.1 (35.6 – 42.4) weeks,

p<0.001) and were less often of Dutch or other Western origin (45% vs. 75%, $\chi 2=638(3)$, p<0.001) than children with behavioral data. Their mothers were 3.0 (95% confidence interval 2.8 – 3.3, t=23.6, p<0.001) years younger, lower educated (20.1% primary level vs. 5.6% primary level, $\chi 2=744(2)$, p<0.001), more often continued smoking during pregnancy (21% vs. 13%, $\chi 2=82(3)$, p<0.001), and had higher scores on psychopathology (median 0.23 (95% range 0 – 1.87) vs. 0.13 (0 – 1.19), p<0.001).

Results

Characteristics of the study subjects are presented in table 1. Children with and without behavioral problems at the age of 18 months differed widely on sociodemographic characteristics. Children within the borderline / clinical range of Total Problems were more often

Table 1. Subject characteristics in children with and without behavioral problems at the age of 18 months

	No behavioral problems N=4259	Borderline or clinical range of Total Problems N= 421	p-value
Male, %	48.9	53.5	0.07
Gestational age at birth, weeks	40.1 (35.7 – 42.4)	40.0 (34.6 – 42.4)	0.07
Preterm birth < 37 weeks, %	4.8	8.4	0.002
Birth weight, g	3453 (559)	3338 (590)	<0.001
Low birth weight < 2500 g, %	5.0	6.9	0.08
National origin			
Dutch / other Western, %	75.1	45.7	
Turkish / Moroccan, %	9.6	22.7	
Surinamese / Antillean, %	7.6	16.9	<0.001
Other non-Western, %	7.7	14.7	
Maternal education			
Primary, %	5.6	18.0	
Secondary, %	37.9	56.0	<0.001
Higher, %	56.5	26.0	
Paternal education			
Primary, %	4.8	13.5	
Secondary, %	35.9	48.4	<0.001
Higher, %	59.3	38.1	
Maternal age at intake, years	31.3 (4.5)	29.1 (5.7)	<0.001
Paternal age at intake, years	33.7 (5.1)	32.5 (6.1)	0.002
GSI score mother in mid-gestation	0.13 (0 – 1.10)	0.31 (0.02 – 1.88)	<0.001
GSI score father in mid-gestation	0.06 (0 – 0.62)	0.12 (0 – 0.96)	<0.001

Values are means ± standard deviations for continuous, normally distributed variables, medians (95% range) for continuous non-normally distributed variables, and percentages for categorical variables. P-values are derived from independent t-tests for continuous normally distributed variables, Mann-Whitney U tests for continuous non-normally distributed variables, or chi-square tests for categorical variables.

GSI = Global Severity Index of psychiatric symptoms

Table 2. Parental characteristics associated with maternal smoking

	Never smoked N = 2829	Quit smoking before pregnancy N = 856	Quit smoking in first trimester N = 387	Continued smoking N = 608	Test statistic (df)	p-value
Maternal education						
Primary, %	5.3	3.3	4.9	10.6		
Secondary, %	33.3	36.8	39.6	60.2	218 (6)	< 0.001
Higher, %	61.4	59.9	55.4	29.2		
Paternal education						
Primary, %	4.2	4.3	3.3	10.4		
Secondary, %	31.6	35.6	40.7	55.8	138 (6)	< 0.001
Higher, %	64.2	60.2	56.0	33.8		
GSI score mother in mid-gestation	0.12 (0 – 1.08)	0.13 (0 – 1.11)	0.15 (0 – 1.13)	0.23 (0 – 1.67)	115 (3)	< 0.001
GSI score father in mid-gestation	0.06 (0 – 0.63)	0.06 (0 – 0.59)	0.05 (0 – 0.74)	0.08 (0 – 0.70)	16.7 (3)	0.001
Paternal smoking, %	33.7	44.6	67.0	74.2	420 (3)	< 0.001
Family net month income						
< 1200 euro	10.0	9.8	12.3	23.0		
1200 – 2000 euro	14.0	14.4	14.9	24.1	132 (6)	< 0.001
> 2000 euro	76.0	75.8	72.8	52.9		
Single mother, %	6.4	7.7	12.3	20.6	127 (3)	< 0.001
Delinquent past mother, %	1.4	2.5	5.2	9.1	114 (3)	< 0.001
Delinquent past father, %	8.0	9.4	16.9	15.1	52 (3)	< 0.001

Values are percentages for categorical variables and medians (95% range) for continuous, non-normally distributed psychopathology scores.

GSI = Global Severity Index of psychiatric symptoms

Test statistic for categorical variables χ^2, and for non-normally distributed continuous variables Kruskal-Wallis.

preterm, had on average lower birth weight, and were more often of non-Western origin than children in the normal range of total problems. Parents of children with behavioral problems were younger and lower educated.

Table 2 compares parental characteristics in the different smoking categories. Mothers who continued smoking during pregnancy had the lowest socioeconomic position, the highest psychopathology scores, were more often single, and had more often a delinquent past than non-smokers.

Table 3 shows the association between maternal smoking and offspring behavioral problems on the CBCL broadband scales, only adjusted for age and gender of the child. Children of mothers who continued smoking during pregnancy had a higher risk (OR 1.6, 95% confidence interval 1.2 – 2.1) of a borderline / clinical score on Total Problems and on Externalizing problems, compared to children of mothers who never smoked.

Next, we examined whether we could identify factors that explain the association between maternal smoking and child behavioral problems (table 4). Model 1 is identical to the model for Total Problems presented in table 3. Model 2 introduces national origin. The higher risk

Table 3. Associations between maternal smoking and child behavioral problems, adjusted for age and gender

Maternal smoking habits		CBCL Broadband scales					
		Total Problems		Internalizing		Externalizing	
	n	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Never smoked	2859	*Reference*		*Reference*		*Reference*	
Quit before pregnancy	856	0.70 (0.56 – 1.01)	0.06	0.82 (0.60 – 1.12)	0.21	0.90 (0.71 – 1.14)	0.39
Quit in first trimester	387	0.84 (0.56 – 1.25)	0.39	0.69 (0.43 – 1.10)	0.21	0.98 (0.71 – 1.35)	0.88
Continued smoking	608	1.59 (1.21 – 2.08)	0.001	1.32 (0.97 – 1.80)	0.07	1.45 (1.15 – 1.84)	0.002

OR = Odds Ratio, CI = Confidence Interval, p = p-value

CBCL Broadband scales were dichotomized using the borderline cut-off score (83rd percentile) of a Dutch norm group.

of behavioral problems in children of mothers who continued smoking remained significant, albeit reduced to 1.5 (95% confidence interval 1.2 – 2.0). Model 3 shows that parental educational attainment and family income reduced the effect of maternal smoking on behavioral problems to an odds ratio of 1.2 (0.9 – 1.6), which was no longer significant. Finally, we introduced parental psychopathology in model 4, and show that these variables in combination accounted entirely for the effect of maternal smoking during pregnancy on child behavior. Marital status and parental criminal record, although related to maternal smoking, did not further change the effect estimates. Similarly, other potential confounders like maternal alcohol use and breastfeeding did not change the effect estimates of model 4. None of the interactions between smoking and the explanatory variables was significant. Birth weight and gestational age at birth, by itself related to behavioral outcome, did not mediate the effect of maternal smoking habits on problem behavior (data not shown).

Similarly, we examined the relationship of intensity of smoking during pregnancy with a high score on the CBCL Total Problems scale (table 5). In the model which was only adjusted for age and gender, children of mothers who smoked > 9 cigarettes a day had an odds ratio of 1.6 (95% confidence interval 1.1 – 2.4) for having behavioral problems at age 18 months compared to mothers who did not smoke (model 1). National origin did not confound the association between level of smoking and children's behavior (model 2). Rather, parental socio-economic status related variables and parental psychopathology scores accounted for the higher risk of behavioral problems with all levels of smoking (models 3 and 4).

Finally, we present the influence of smoking by father during pregnancy on child behavioral outcome in table 6. Passive smoking of the pregnant woman, due to smoking indoors by father, was related to a higher risk of behavioral problems. The effect of passive smoking was similar to the effect of active smoking (OR of Total Problems with active smoking of the mother as a reference: 0.99 (95% confidence interval 0.68 – 1.45), p = 0.98). When father smoked outside, there was no increased risk of behavioral problems. The odds of Total Problems for children of fathers who smoked indoors was 72% higher (OR 1.72, 95% confidence interval 1.18 – 2.49, p = 0.005) than for children of fathers who smoked outside during pregnancy. None of the effects of parental smoking during pregnancy on behavioral outcome remained

Table 4. Successive models of the effects of maternal smoking on total behavioral problems

	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Maternal smoking								
Never smoked	*Reference*		*Reference*		*Reference*		*Reference*	
Quit before pregnancy	0.75 (0.56 – 1.01)	0.06	0.82 (0.60 – 1.12)	0.22	0.81 (0.59 – 1.12)	0.81	0.80 (0.58 – 1.10)	0.17
Quit in first trimester	0.84 (0.56 – 1.25)	0.39	0.98 (0.65 – 1.47)	0.92	0.88 (0.58 – 1.33)	0.88	0.86 (0.57 – 1.31)	0.48
Continued smoking	1.59 (1.22 – 2.08)	0.001	1.52 (1.15 – 2.01)	0.003	1.19 (0.89 – 1.60)	0.24	1.05 (0.78 – 1.42)	0.74
National origin								
Dutch / other Western	-		*Reference*		*Reference*		*Reference*	
Turkish / Moroccan	-		4.18 (3.18 – 5.51)	< 0.001	2.06 (1.47 – 2.88)	< 0.001	1.80 (1.27 – 2.54)	0.001
Surinamese / Antillean	-		3.41 (2.49 – 4.67)	< 0.001	2.09 (1.48 – 2.95)	< 0.001	2.05 (1.44 – 2.90)	< 0.001
Other non-Western	-		3.55 (2.60 – 4.85)	< 0.001	2.20 (1.56 – 3.11)	< 0.001	2.13 (1.50 – 3.02)	< 0.001
Maternal education								
Primary	-		-		1.82 (1.22 – 2.72)	0.004	1.91 (1.27 – 2.88)	0.002
Secondary	-		-		1.16 (0.89 – 1.52)	0.27	1.12 (0.85 – 1.47)	0.48
Higher	-		-		*Reference*		*Reference*	
Paternal education								
Primary	-		-		1.49 (0.89 – 2.49)	0.13	1.36 (0.81 – 2.29)	0.25
Secondary	-		-		1.48 (1.09 – 2.00)	0.01	1.41 (1.04 – 1.94)	0.03
Higher	-		-		*Reference*		*Reference*	
Family income								
< 1200 euros	-		-		2.42 (1.73 – 3.39)	< 0.001	1.89 (1.33 – 2.68)	< 0.001
1200 – 2000 euros	-		-		1.33 (0.96 – 1.85)	0.09	1.16 (0.83 – 1.63)	0.38
> 2000 euros	-		-		*Reference*		*Reference*	
Maternal GSI (per SD)	-		-		-		1.32 (1.22 – 1.43)	< 0.001
Paternal GSI (per SD)	-		-		-		1.13 (1.04 – 1.23)	0.006

OR = Odds Ratio, CI = Confidence Interval, SD = standard deviation, GSI = Global Severity Index of psychiatric symptoms.

Model 1 = adjusted for age and gender, Model 2 = as model 1, additionally adjusted for national origin, Model 3 = as model 2, additionally adjusted for parental education and family income, Model 4 = as model 3, additionally adjusted for parental psychiatric symptoms.

Table 5. Number of cigarettes per day smoked during pregnancy and offspring's total behavioral problems

Maternal smoking habits		Model 1	Model 2	Model 3	Model 4
	n	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
None	3664	*Reference*	*Reference*	*Reference*	*Reference*
< 5 cigarettes a day	512	1.28 (0.95 – 1.74)	1.25 (0.91 – 1.72)	1.09 (0.79 – 1.51)	0.99 (0.71 – 1.37)
5 – 9 cigarettes a day	272	1.42 (0.96 – 2.10)	1.43 (0.95 – 2.14)	1.09 (0.72 – 1.66)	1.03 (0.67 – 1.57)
> 9 cigarettes a day	232	1.58 (1.05 – 2.38)	1.61 (1.05 – 2.47)	1.26 (0.81 – 1.95)	1.16 (0.74 – 1.81)
P for trend		0.004	0.005	0.28	0.60

OR = Odds Ratio, CI = Confidence Interval

Model 1 = adjusted for age and gender

Model 2 = as model 1, additionally adjusted for national origin

Model 3 = as model 2, additionally adjusted for parental education and family income

Model 4 = as model 3, additionally adjusted for parental psychiatric symptoms.

Table 6. Associations between parental smoking during mid- and late pregnancy and child behavioral problems

Parental smoking habits		CBCL Broadband scales, associated for age and gender					
		Total Problems		Internalizing		Externalizing	
Model 1	n	OR (95% CI)	P	OR (95% CI)	p	OR (95% CI)	p
No active or passive smoking	2205	*Reference*		*Reference*		*Reference*	
Father smoked outside, mother did not smoke	998	1.15 (0.86 – 1.52)	0.35	1.05 (0.77 – 1.44)	0.74	1.23 (0.98 – 1.55)	0.07
Father smoked indoors, mother did not smoke	397	1.97 (1.40 – 2.76)	< 0.001	1.78 (1.23 – 2.59)	0.002	1.66 (1.24 – 2.23)	0.001
Mother smoked actively	608	1.98 (1.48 – 2.64)	< 0.001	1.60 (1.15 – 2.23)	0.005	1.67 (1.30 – 2.15)	< 0.001
		CBCL Broadband scales, fully adjusted					
		Total Problems		Internalizing		Externalizing	
Model 2	n	OR (95% CI)	P	OR (95% CI)	p	OR (95% CI)	p
No active or passive smoking	2205	*Reference*		*Reference*		*Reference*	
Father smoked outside, mother did not smoke	998	1.16 (0.87 – 1.56)	0.32	1.10 (0.79 – 1.53)	0.57	1.24 (0.98 – 1.56)	0.07
Father smoked indoors, mother did not smoke	397	1.19 (0.82 – 1.71)	0.36	1.04 (0.69 – 1.57)	0.86	1.26 (0.92 – 1.73)	0.14
Mother smoked actively	608	1.22 (0.89 – 1.69)	0.22	0.99 (0.69 – 1.44)	0.97	1.23 (0.94 – 1.61)	0.14

OR = Odds Ratio, CI = Confidence Interval

CBCL Broadband scales were dichotomized using the borderline cut-off score (83rd percentile) of a Dutch norm group.

Model 1 is only adjusted for age and gender of the child.

Model 2 is additionally adjusted for parental educational level, family income, national origin, and parental psychopathology.

Odds ratios of active maternal smoking differ from odds ratios in table 3, due to a changed reference category.

significant after adjustment for parental characteristics (models 2 of table 6). We repeated the analyses with only fathers who provided information on smoking habits themselves, which yielded similar results.

Discussion

The present study showed that children of mothers who continued smoking during pregnancy had a higher risk of behavioral problems, compared to children of non-smoking mothers. However, the observed association between maternal smoking during pregnancy and children's behavior was accounted for entirely by national origin, parental socioeconomic status and parental psychiatric symptoms. In line with this, the dose-response relationship of maternal smoking, and the effects of smoking by father were explained by parental socioeconomic status and psychopathology as well.

Several reviews suggested small but significant associations between maternal smoking during pregnancy and behavioral problems in the offspring [5-7, 13]. However, these reviews also concluded that results are difficult to interpret because of the numerous confounding variables [5, 13]. In particular, Linnet et al [6] reported that 'information on parental psychopathology is essential to fully exploring the association between ADHD and exposure to maternal lifestyle factors'. Other main potential confounders in studies of cigarette smoke exposure are, according to these reviews, parental socioeconomic status, home environment, other environmental or personal exposures, child-rearing practices, and parental intelligence [13].

Earlier studies in large (n>1000) population-based samples that prospectively assessed smoking habits of the pregnant women described effects of maternal smoking during pregnancy on externalizing behavior [14] and hyperactivity [15] in childhood and violent offending in adulthood [16, 17]. These studies adjusted the association for socioeconomic status, which, despite reduced risk ratios, did not account for the significant associations. Very few researchers, such as Eskenazi and Trupin [18], described complete attenuation in the association between prenatal smoke exposure and active behavior after adjustment for socioeconomic status. Brennan et al [17] and Linnet et al [19] adjusted for psychiatric hospitalization as a proxy of psychopathology, which did not explain the effect of maternal smoking on offspring behavior, whereas others assessed only one or two specific psychiatric disorders [7, 14, 20-24]. Although most studies found no complete attenuation of the relationship between maternal smoking in pregnancy and behavioral problems, two studies in large twin samples showed that effect estimates of prenatal smoking on conduct disorders were greatly reduced if paternal antisocial personality disorder and maternal depression were controlled for [22, 23]. Few studies used continuous variables of psychiatric symptoms in the analyses [21, 22, 24]. Our study shows, by providing both maternal and paternal information on psychiatric symptoms throughout a range of different disorders, that measurement of parental psychopathology is important to interpret the association between prenatal smoke exposure and behavioral disorders.

Our findings are in line with studies that described confounding in the relationship between maternal smoking in pregnancy and cognitive performance in childhood [25] and

adolescence [26]. These studies concluded that the effects of prenatal cigarette smoke exposure on cognitive function could well be entirely explained by characteristics of the home environment and maternal cognitive abilities.

Discrepancies in the confounding effects of social class in the studies so far may be the result of varying patterns of tobacco consumption among countries, cultures and time [27]. It is known that health-related behaviors like smoking changed from typical of men in advantaged classes to a higher prevalence in disadvantaged classes. Since women lag 10 – 20 years behind men, these trends in social inequalities in smoking habits are probably still ongoing [28].

Information on the effects of passive smoking of the mother during pregnancy is scarce and inconsistent. Eskenazi and Trupin [18] found no relation between passive smoking during pregnancy and cognitive development or activity level. Others described similar effects of active and passive prenatal cigarette smoke exposure on cognition [29] and behavior [30] in children. Finally, the findings of Makin et al [31] suggest a dose-response relationship of passive and active cigarette smoke exposure on the fetus. We show that children of fathers who smoked in the same room as their non-smoking pregnant partner have a higher risk of behavioral problems compared to children of non-smokers. This effect of passive smoking did not differ from the effect of active smoking. Indoor paternal smoking during pregnancy is, like active maternal smoking during pregnancy, likely to be a vulnerability marker for child behavioral and emotional problems. Our finding that smoking outside by father was not related to behavioral problems suggests that these fathers take the health of their pregnant partner into account, and thereby create a more favorable environment for their unborn child than fathers who smoked in the same room as their pregnant wives.

A strength of our study was the assessment of maternal smoking habits during pregnancy at several time-points during pregnancy, which enables identification of women who quit during pregnancy and makes classification of smoking during vulnerability periods more precise. Several limitations need to be discussed as well. First, selective attrition may have influenced our findings. Children and mothers without information on behavioral outcome differed from families with behavioral information on both maternal smoking and several important confounding variables. We cannot infer from our data whether the association between maternal smoking in pregnancy and behavioral problems in nonparticipating children would not have been confounded by socioeconomic status and parental psychopathology. The use of self-reports of maternal smoking during pregnancy is a second potential limitation. Another source of reporter bias is the use of a parent report to assess behavioral problems. Parental perception of problems might lead to misclassification, which, in theory, could be related to their smoking habits. Our adjustment for maternal symptoms of psychopathology may capture part of a possible information bias, but only studies using several observers of behavior can clarify this issue. Finally, we may have overadjusted our associations by control-

ling for socioeconomic status that may, in part act as a preceding factor in the association between smoking and behavioral problems.

Our findings provide new information on the phenomenon of maternal smoking during pregnancy in relation to behavioral problems. Maternal, and also paternal, smoking habits are mainly markers for a set of vulnerabilities for child behavioral and emotional problems. We found no indication that smoking is causally related to behavioral disorders via disturbances in intra-uterine growth or brain development. Notwithstanding our results, it may well be that prenatal cigarette smoke exposure is related to behavioral problems in the most vulnerable children via gene-environment interactions. Furthermore, there is wide consensus that maternal smoking in pregnancy has adverse effects on the pregnant woman as well as on perinatal morbidity and mortality in the child. These negative effects of prenatal nicotine exposure, as well as the high prevalence of parental smoking, underscore the importance of developing programs aimed at smoking prevention and cessation in pregnant women and their partners. These programs need to be accompanied by assessment of and intervention strategies to vulnerabilities that are highly related to smoking habits, such as parental psychopathology.

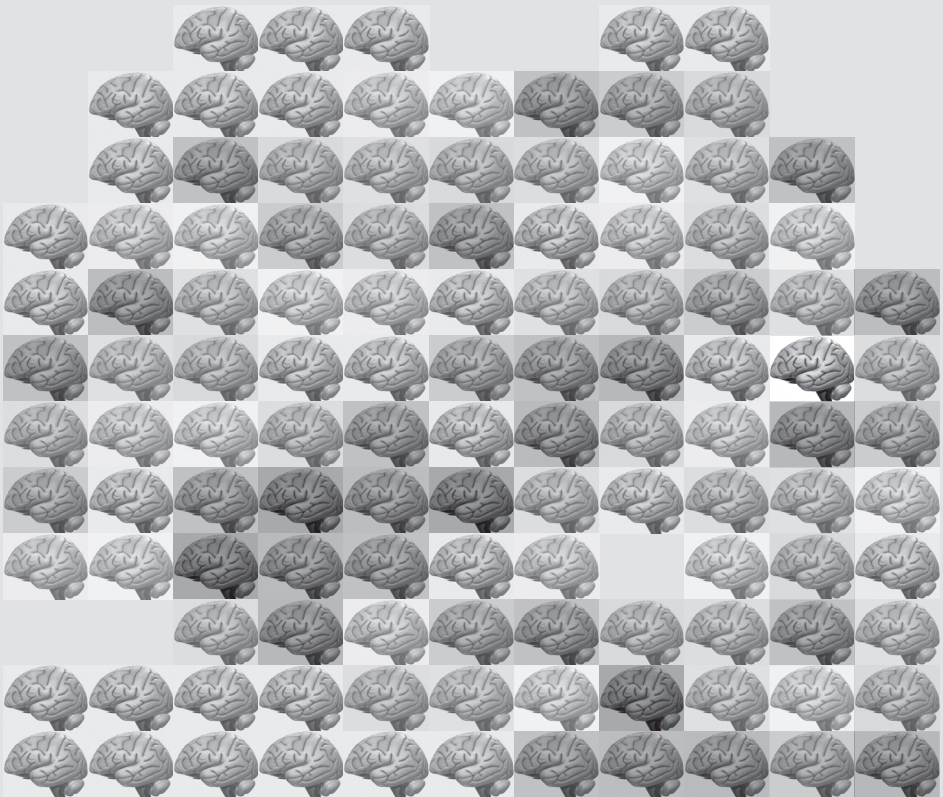
References

1. Abel EL. Smoking during pregnancy: a review of effects on growth and development of offspring. Hum Biol 1980 Dec;52(4): 593-625.
2. Lieberman E, Gremy I, Lang JM, Cohen AP. Low birthweight at term and the timing of fetal exposure to maternal smoking. Am J Public Health 1994 Jul;84(7): 1127-31.
3. Slotkin TA. Fetal nicotine or cocaine exposure: which one is worse? J Pharmacol Exp Ther 1998 Jun;285(3): 931-45.
4. Perkins KA, Sexton JE, DiMarco A, Fonte C. Acute effects of tobacco smoking on hunger and eating in male and female smokers. Appetite 1994 Apr;22(2): 149-58.
5. Ernst M, Moolchan ET, Robinson ML. Behavioral and neural consequences of prenatal exposure to nicotine. J Am Acad Child Adolesc Psychiatry 2001 Jun;40(6): 630-41.
6. Linnet KM, Dalsgaard S, Obel C, et al. Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: review of the current evidence. Am J Psychiatry 2003 Jun;160(6): 1028-40.
7. Wakschlag LS, Pickett KE, Cook E, Jr., Benowitz NL, Leventhal BL. Maternal smoking during pregnancy and severe antisocial behavior in offspring: a review. Am J Public Health 2002 Jun;92(6): 966-74.
8. Jaddoe VW, Mackenbach JP, Moll HA, et al. The Generation R Study: Design and cohort profile. Eur J Epidemiol 2006;21(6): 475-84.
9. Achenbach TM, Rescorla LA. Manual for the ASEBA Preschool Forms & Profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families; 2000.
10. Tick NT, van der Ende J, Koot HM, Verhulst FC. 14-year changes in emotional and behavioral problems of very young Dutch children. J Am Acad Child Adolesc Psychiatry 2007 Oct;46(10): 1333-40.
11. de Beurs E. Brief Symptom Inventory, handleiding. Leiden, the Netherlands; 2004.
12. Derogatis LR, Melisaratos N. The Brief Symptom Inventory: an introductory report. Psychol Med 1983 Aug;13(3): 595-605.
13. Eskenazi B, Castorina R. Association of prenatal maternal or postnatal child environmental tobacco smoke exposure and neurodevelopmental and behavioral problems in children. Environ Health Perspect 1999 Dec;107(12): 991-1000.
14. Williams GM, O'Callaghan M, Najman JM, et al. Maternal cigarette smoking and child psychiatric morbidity: a longitudinal study. Pediatrics 1998 Jul;102(1): e11.
15. Kotimaa AJ, Moilanen I, Taanila A, et al. Maternal smoking and hyperactivity in 8-year-old children. J Am Acad Child Adolesc Psychiatry 2003 Jul;42(7): 826-33.
16. Rasanen P, Hakko H, Isohanni M, Hodgins S, Jarvelin MR, Tiihonen J. Maternal smoking during pregnancy and risk of criminal behavior among adult male offspring in the Northern Finland 1966 Birth Cohort. Am J Psychiatry 1999 Jun;156(6): 857-62.
17. Brennan PA, Grekin ER, Mednick SA. Maternal smoking during pregnancy and adult male criminal outcomes. Arch Gen Psychiatry 1999 Mar;56(3): 215-9.
18. Eskenazi B, Trupin LS. Passive and active maternal smoking during pregnancy, as measured by serum cotinine, and postnatal smoke exposure. II. Effects on neurodevelopment at age 5 years. Am J Epidemiol 1995 Nov 1;142(9 Suppl): S19-29.
19. Linnet KM, Wisborg K, Obel C, et al. Smoking during pregnancy and the risk for hyperkinetic disorder in offspring. Pediatrics 2005 Aug;116(2): 462-7.
20. Whitaker RC, Orzol SM, Kahn RS. Maternal mental health, substance use, and domestic violence in the year after delivery and subsequent behavior problems in children at age 3 years. Arch Gen Psychiatry 2006 May;63(5): 551-60.
21. Leech SL, Richardson GA, Goldschmidt L, Day NL. Prenatal substance exposure: effects on attention and impulsivity of 6-year-olds. Neurotoxicol Teratol 1999 Mar-Apr;21(2): 109-18.

22. Maughan B, Taylor A, Caspi A, Moffitt TE. Prenatal smoking and early childhood conduct problems: testing genetic and environmental explanations of the association. Arch Gen Psychiatry 2004 Aug;61(8): 836-43.
23. Silberg JL, Parr T, Neale MC, Rutter M, Angold A, Eaves LJ. Maternal smoking during pregnancy and risk to boys' conduct disturbance: an examination of the causal hypothesis. Biol Psychiatry 2003 Jan 15;53(2): 130-5.
24. Huijbregts SC, Seguin JR, Zoccolillo M, Boivin M, Tremblay RE. Associations of maternal prenatal smoking with early childhood physical aggression, hyperactivity-impulsivity, and their co-occurrence. J Abnorm Child Psychol 2007 Apr;35(2): 203-15.
25. Breslau N, Paneth N, Lucia VC, Paneth-Pollak R. Maternal smoking during pregnancy and offspring IQ. Int J Epidemiol 2005 Oct;34(5): 1047-53.
26. Lambe M, Hultman C, Torrang A, Maccabe J, Cnattingius S. Maternal smoking during pregnancy and school performance at age 15. Epidemiology 2006 Sep;17(5): 524-30.
27. Huisman M, Kunst AE, Mackenbach JP. Inequalities in the prevalence of smoking in the European Union: comparing education and income. Prev Med 2005 Jun;40(6): 756-64.
28. Borrell C, Rue M, Pasarin MI, Rohlfs I, Ferrando J, Fernandez E. Trends in social class inequalities in health status, health-related behaviors, and health services utilization in a Southern European urban area (1983-1994). Prev Med 2000 Dec;31(6): 691-701.
29. Rantakallio P. A follow-up study up to the age of 14 of children whose mothers smoked during pregnancy. Acta Paediatr Scand 1983 Sep;72(5): 747-53.
30. Gatzke-Kopp LM, Beauchaine TP. Direct and passive prenatal nicotine exposure and the development of externalizing psychopathology. Child Psychiatry Hum Dev 2007 Dec;38(4): 255-69.
31. Makin J, Fried PA, Watkinson B. A comparison of active and passive smoking during pregnancy: long-term effects. Neurotoxicol Teratol 1991 Jan-Feb;13(1): 5-12.

2.4

Maternal folic acid use in early pregnancy and child behavior problems



Abstract

Background: Folate deficiency during embryogenesis is an established risk-factor for neural tube defects in the fetus. An adequate folate nutritional status is also important for normal fetal growth and brain development. Whether folic acid use of the mother during pregnancy is related to behavioral development is less clear.

Methods: Within a population-based cohort, we prospectively assessed folic acid supplement use during the first trimester by questionnaire. Child behavioral and emotional problems were assessed with the Child Behavior Checklist at the age of 18 months in 4,214 toddlers.

Results: Children of mothers who did not use folic acid supplements in the first trimester had a higher risk of Total Problems (OR 1.44, 95% confidence interval: 1.12 – 1.86). Folic acid supplement use protected both from Internalizing (OR of no supplement use: 1.65, 95% confidence interval 1.24 – 2.19) and Externalizing problems (OR 1.45, 95% confidence interval 1.17 – 1.80), even when adjusted for maternal characteristics. Birth weight and size of the fetal head did not mediate the association between folic acid use and child behavior.

Conclusions: Inadequate use of folic acid supplements during early pregnancy is associated with a higher risk of behavioral problems in the offspring. These findings underscore the importance of folic acid supplementation in pregnant women. This preventive strategy may also reduce mental health problems in children.

Introduction

Folic acid, an essential micronutrient, is involved in synthesis of DNA, RNA, and proteins [1]. A deficiency in folate during pregnancy is a well-known risk factor for neural tube defects [2, 3]. Moreover, an adequate folate status is important for normal fetal growth [4]. Animal studies have established essential roles for folates in the development of the central nervous system as well. Folates influence neuronal and glial growth and proliferation, and are indirectly involved in the synthesis of neurotransmitters, such as dopamine, norepinephrine, epinephrine, and serotonin [5, 6].

Information on the association between prenatal folate status and human neurodevelopment after closure of the neural tube is scarce and inconclusive. Following the reported coincidence of an increased risk of schizophrenia with an increased risk of neural tube defects in mothers exposed to severe famine during the preconceptional period, it has been hypothesized that maternal low folate is a risk factor for developing schizophrenia [7]. Brown et al. found an association between maternal hyperhomocysteinemia, which is inversely related to folate, in the third trimester of pregnancy, and the risk of adult schizophrenia [8]. Several inborn errors of folate mechanism give rise to mental retardation and autistic symptoms [9, 10]. On the other hand, Tamura et al. found no differences in neuropsychological test scores between children exposed to low and high folates in pregnancy [11]. Whether maternal folate deficiencies are related to behavioral problems in the offspring, is yet unclear.

The present study examines the association between folic acid use during the first trimester of pregnancy and child behavioral and emotional problems. We hypothesized that inadequate folic acid supplement use during embryogenesis was related to a higher risk of behavioral problems in the offspring.

Materials and methods

Setting

This study was conducted within the Generation R Study, a population-based cohort in Rotterdam, the Netherlands [12, 13]. Enrolment was aimed in early pregnancy. All children were born between April 2002 and January 2006 and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all adult participants.

Study population

In total, 7654 live born children and their mothers, who were prenatally included in Generation R, were approached for postnatal consent. Of these, mothers without information on folic acid supplement use in early pregnancy were excluded (n= 1139, 14.9 %). Twenty-four children deceased in the first few months after birth. The remaining 6491 children were eligible for the present study. Mothers of 788 children refused full consent for postnatal participation. Another 1489 mothers did not complete the 18-month-questionnaire. Information on child behavioral problems at age 18 months was available in 4214 toddlers (64.9% of 6491). Some mothers participated with two children (n=268), and another four mothers participated with three children. Since results did not differ after random exclusion of one or two of these siblings, they were included in the analyses.

Maternal folic acid use

Information on folic acid use as a single or multivitamin preparation in the first trimester was obtained by questionnaire in early pregnancy (gestational age < 18 weeks). Pregnant women were asked whether they used folic acid supplements and when supplementation was started (before pregnancy, as soon as pregnancy was known, later in pregnancy). Since results did not differ between women who started folic acid supplement use preconceptionally and women who started within the first 10 weeks of pregnancy, we categorized folic acid supplement use into 'preconceptional start or start during the first 10 weeks of pregnancy' and 'no use during embryogenesis'.

Child behavioral and emotional problems

The Child Behavior Checklist for toddlers (CBCL/1½-5) was used to obtain standardized parent reports of children's problem behaviors. The CBCL/1½-5 contains 99 problem items, which are scored on seven empirically based syndromes that were derived by factor analyses: Emotionally Reactive, Anxious/Depressed, Somatic Complaints, Withdrawn, Sleep Problems, Attention Problems, and Aggressive Behavior. The broadband scale Internalizing is the sum score of items in the first four syndrome scales, whereas Externalizing is the sum score of Attention Problems and Aggressive Behavior. Total Problems is the sum score of all 99 problem items. Each item is scored 0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true, based on the preceding two months. Good reliability and validity have been reported for the CBCL [14]. We used the borderline cut-off scores (83rd percentile for the broadband scales and 93rd percentile for the syndrome scales of a Dutch norm group [15]) to classify children as having behavioral problems in the borderline / clinical range.

Covariates

Date of birth, birth weight, birth order, and gender of the infant were obtained from midwife and hospital registries at birth. We established gestational age by using the fetal ultrasound examinations within the Generation R Study. Head circumference, biparietal diameter, transcerebellar diameter, and atrial width of lateral ventricle were assessed by fetal ultrasound in late pregnancy (median gestational age 30 weeks, 95% range 28 - 33 weeks) using standardized ultrasound protocols. Information about maternal age, educational level, national origin, maternal psychopathology, and pregnancy planning was obtained by questionnaire. The highest completed education (primary school, secondary school, and higher education) decided on the educational level of the mother. The pregnant woman was of non-Dutch origin if one of her parents was born abroad. If both parents of the participating mother were born abroad, we used the country of birth of the mother of mother to classify the ethnic background. We classified national origin into five categories: a) Dutch, b) other Western, c) Turkish or Moroccan, d) Surinamese or Antillean, or e) other non-Western. To assess maternal and paternal psychopathology in mid-pregnancy (gestational age 18 - 25 weeks), we used the Brief Symptom Inventory (BSI) [16, 17]. The score on the Global Severity Index was standardized and used as a continuous variable. Maternal smoking and maternal alcohol use were assessed at three time-points during pregnancy (early pregnancy, mid-pregnancy, and late pregnancy) and categorized into 'yes, during pregnancy' and 'no use during pregnancy'. We measured weight and height of the pregnant woman three times in pregnancy during visits at the research center. Body mass index was calculated by dividing weight through squared height. Breastfeeding was assessed at 2, 6, and 12 months of age. We categorized breastfeeding into 'stopped before 3 months of age' and 'continued breastfeeding after 3 months of age'.

Statistical analyses

Differences in baseline characteristics between mothers who used and did not use folic acid supplements were compared with the chi-square statistic for categorical variables, independent t-tests for continuous normally distributed variables, and Mann-Whitney U tests for continuous non-normally distributed variables. Successive logistic regression models are presented to show whether the association between maternal folic acid use and behavioral problems holds-up when potential confounders are controlled. Folic acid supplement use was the reference category in these analyses, and the odds ratios represent a higher risk of behavioral problems in children of mothers who did not use folic acid supplements. We interpret these odds ratios as a lower risk of behavioral problems due to folic acid supplement use. Confounders were included in the final models if the effect estimate of folic acid use changed meaningfully (defined as more than 5%). Interaction terms of folic acid use with all confound-

ing variables were tested and included at α = 0.15. Measures of association are presented with their 95% confidence intervals (CI). Statistical analyses were carried out using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

Response analyses

Analyses of missing data showed that children without information on behavior had on average 110 (95% confidence interval 81 – 139, t=7.4, p<0.001) grams lower birth weight, and had shorter gestation (median 40.0 (95% range 34.9 – 42.3) weeks vs. 40.1 (35.6 – 42.4) weeks, p<0.001) than children with behavioral data. Their mothers were on average 3.0 (95% confidence interval 2.8 – 3.3, t=22.4, p<0.001) years younger, lower educated (19.9 % primary level vs. 5.7%, χ^2=714(2), p<0.001), and less often of Dutch origin (29.6% vs. 63.3%, χ^2=858(4), p<0.001) than mothers of children with behavioral data. They less often used folic acid supplements during the first trimester (44.9% vs. 72.8%, χ^2=526(1), p<0.001).

Results

A description of the participating women and their children is presented in table 1. Several child and maternal characteristics differed widely between mothers who used folic acid during embryogenesis and mothers who did not. Mothers who used folic acid were more often of Dutch origin, higher educated, were older, and had lower psychopathology scores than mothers who did not used folic acid (table 1). Folic acid supplementation during pregnancy was highly related to lifestyle factors as well, which can be seen in the differences in smoking, alcohol use, and maternal body mass index. The children of mothers who used folic acid had higher birth weight, larger head circumference and larger ventricular size in late pregnancy than children of mothers who did not use folic acid (table 1).

Table 2 shows the association between maternal folic acid use during the first trimester and the CBCL Total Problems score at age 18 months. Model 1 of table 2 was only adjusted for age and gender of the child. The odds ratio for behavioral problems in children of mothers who did not use folic acid supplements during embryogenesis was 2.8 (95% confidence interval 2.2 – 3.4) compared to children of mothers who used folic acid supplements. Model 2 introduces several important confounding variables in the association of folic acid supplementation and child behavioral problems. The higher risk of behavioral problems in children of mothers who did not use folic acid supplements, albeit reduced to 1.4 (95% confidence interval 1.1 – 1.9), remained statistically significant after adjustment for maternal age, educational level, national origin, and psychopathology. Other maternal characteristics such as pregnancy planning, maternal smoking during pregnancy, maternal alcohol use during pregnancy, maternal body mass index and breastfeeding, did not further confound

Table 1. Subject characteristics in offspring of mothers using folic acid supplementation during embryogenesis

	Folic acid use	No folic acid use	p-value
	N= 3,067	N= 1,147	
Male, %	48.7	50.5	0.30
Gestational age at birth, weeks	40.1 (35.6 – 42.4)	40.0 (36.0 – 42.3)	<0.001
Preterm birth < 37 weeks, %	5.1	4.4	0.42
Birth weight, g	3493 (545)	3384 (546)	<0.001
Low birth weight < 2500 g, %	3.6	5.6	0.004
Head circumference in late pregnancy, mm	286.3 (12.0)	284.9 (12.0)	0.001
Transcerebellar diameter in late pregnancy, mm	37.9 (2.4)	37.8 (2.5)	0.77
Atrial width of lateral ventricle in late pregnancy, mm	5.0 (1.7)	4.8 (1.7)	0.001
National origin			
Dutch, %	71.8	40.1	
Other Western, %	13.3	12.6	
Turkish / Moroccan, %	5.1	20.8	<0.001
Surinamese / Antillean, %	5.3	14.2	
Other non-Western, %	4.6	12.3	
Maternal education			
Primary, %	2.5	14.0	
Secondary, %	34.0	47.3	<0.001
Higher, %	63.5	38.7	
Maternal smoking during pregnancy, % yes	20.1	26.6	<0.001
Maternal alcohol use during pregnancy, % > 1 glass/week	26.1	22.8	0.03
Pregnancy planned, % yes	86.4	57.1	<0.001
Maternal age at intake, years	31.7 (4.2)	29.7 (5.4)	<0.001
BMI mother at intake, kg/m^2	24.2 (4.0)	24.8 (4.6)	<0.001
GSI score mother in mid-gestation	0.12 (0 – 0.96)	0.19 (0 – 1.61)	<0.001

Values are means ± standard deviations for continuous, normally distributed variables, medians (95% range) for continuous non-normally distributed variables, and percentages for categorical variables. P-values are derived from independent t-tests for continuous normally distributed variables, Mann-Whitney U tests for continuous non-normally distributed variables, or chi-square tests for categorical variables.

GSI = Global Severity Index of psychiatric symptoms

the effect of folic acid use on behavioral problems. Model 3 and 4 of table 2 show whether the association between folic acid use and offspring behavior was mediated by fetal growth. Birth weight and head circumference were not related to behavioral outcome after adjustment for maternal characteristics and did therefore not mediate the association between folic acid use and child behavior. Gestational age at birth was related to behavioral outcome (OR 0.9 (95% confidence interval 0.9 – 1.0)), but did not change the effect estimate of folic acid use on behavioral outcome. Similarly, transcerebellar diameter and atrial width of the lateral ventricle during late pregnancy did not explain the association between folic acid use and child behavioral problems (OR after adjustment for these factors 1.4 (95% confidence interval 1.1 – 1.9, p = 0.004)). None of the interaction terms between folic acid use and the explanatory variables was significant at α = 0.15.

Table 2. Maternal folic acid use and child behavioral problems (Total Problems score)

	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)	Model 4 OR (95% CI)
Folic acid use				
Supplement use during the first 10 weeks of pregnancy	*Reference*	*Reference*	*Reference*	*Reference*
No supplement use	2.78 (2.24 – 3.44) p < 0.001	1.44 (1.12 – 1.86) p = 0.005	1.44 (1.12 – 1.86) p = 0.005	1.43 (1.11– 1.85) p = 0.005
Maternal age (per year)	-	0.96 (0.94 – 0.98) p = 0.001	0.96 (0.94 – 0.98) p = 0.001	0.96 (0.94 – 0.98) p = 0.001
National origin				
Dutch	-	*Reference*	*Reference*	*Reference*
Other Western	-	1.96 (1.40 – 2.79)	1.97 (1.40 – 2.78)	1.97 (1.40 – 2.78)
Turkish / Moroccan	-	3.07 (2.15 – 4.35)	3.11 (2.19 – 4.42)	3.09 (2.18 – 4.40)
Surinamese / Dutch Antillean	-	2.30 (1.59 – 3.41)	2.27 (1.54 – 3.36)	2.31 (1.56 – 3.40)
Other non-Western	-	3.49 (2.41 – 5.04) p < 0.001†	3.48 (2.40 – 5.04) p < 0.001†	3.49 (2.41 – 5.06) p < 0.001†
Educational level				
Primary	-	1.96 (1.31 – 2.94)	1.96 (1.31 – 2.94)	1.96 (1.31 – 2.94)
Secondary	-	1.15 (0.88 – 1.51)	1.13 (0.86 – 1.48)	1.14 (0.87 – 1.50)
Higher	-	*Reference* p = 0.01†	*Reference* p = 0.01†	*Reference* p = 0.01†
Maternal psychopathology (per SD)	-	1.32 (1.22 – 1.44) p < 0.001	1.32 (1.22 – 1.44) p < 0.001	1.32 (1.22 – 1.44) p < 0.001
Birth weight (per SD)	-	-	0.98 (0.87 – 1.11) p = 0.78	-
Gestational age at birth (per week)	-	-	0.94 (0.89 – 1.00) p = 0.04	-
Head circumference in late pregnancy (per SD)	-	-	-	0.96 (0.85 – 1.08) p = 0.49

CI = Confidence interval, SD = standard deviation, p = p-value.

Model 1 = adjusted for age and gender

Model 2 = model 1, additionally adjusted for maternal characteristics (i.e. maternal age, national origin, educational level, and psychopathology).

Model 3 = model 2, additionally adjusted for birth weight and gestational age at birth

Model 4 = model 2, additionally adjusted for head circumference in late pregnancy.

† Overall p-value for categorical variables.

Table 3 shows the association between folic acid use during the first trimester and the broadband and syndrome scales of the Child Behavior Checklist, after adjustment for maternal characteristics. Children of mothers who did not use folic acid supplements had a higher risk of Internalizing problems (OR 1.7, 95% confidence interval 1.2 – 2.2) and of Externalizing problems (OR 1.5, 95% confidence interval 1.2 – 1.8). When we stratified our sample on the basis of national origin, all Odds Ratios for Externalizing and Internalizing problems were above one. In the Dutch group (n= 2662), the Odds Ratio for Externalizing problems in children of mothers who used folic acid supplements was 1.8 (95% confidence interval 1.4 – 2.5, p < 0.001).

Table 3. Maternal folic acid use supplementation during embryogenesis and child behavioral problems

Child Behavior Checklist broadband scales								
	Internalizing				Externalizing			
Folic acid use	OR (95% CI)		p		OR (95% CI)		p	
Supplement use	*Reference*				*Reference*			
No use	1.65 (1.24 – 2.19)		0.001		1.45 (1.17 – 1.80)		0.001	
Internalizing syndrome scales								
	Emotionally Reactive		Anxious / Depressed		Somatic Complaints		Withdrawn	
Folic acid use	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Supplement use	*Reference*		*Reference*		*Reference*		*Reference*	
No use	1.26 (0.79 – 1.99)	0.33	1.33 (0.88 – 2.01)	0.18	1.32 (0.93 – 1.86)	0.12	1.48 (1.11 – 1.96)	0.007
Externalizing syndrome scales								
	Attention Problems				Aggressive Behavior			
Folic acid use	OR (95% CI)		p		OR (95% CI)		p	
Supplement use	*Reference*				*Reference*			
No use	1.30 (1.03 – 1.64)		0.03		1.51 (1.04 – 2.19)		0.03	

OR = Odds Ratio, CI = Confidence interval, p = p-value

All models were adjusted for age and gender, and for maternal age, maternal national origin, maternal educational level, and maternal psychopathology during pregnancy.

Maternal folic acid use was significantly related to three syndrome scales (table 3). The odds for showing Withdrawn behavior were 48% higher when mothers did not use folic acid supplements in early pregnancy. Second, use of folic acid supplements was related to a lower risk of Attention Problems and Aggressive Behavior. Mothers who started folic acid supplement use after 10 weeks of pregnancy (n=507) had no lower risk of behavioral problems on any of the subscales compared to mothers who never used folic acid supplements during pregnancy (n=640) (data not shown).

Discussion

The present study showed that children of mothers who used folic acid supplements during early pregnancy had a significantly lower risk of behavioral problems, compared to children of mothers who did not use folic acid supplementation. The observed association was confounded by maternal characteristics such as age, educational level, national origin, and maternal psychopathology, but did not disappear after adjustment for the confounders measured. Birth weight and fetal head growth characteristics did not mediate the association between folic acid use and behavioral problems.

The current recommendation regarding folic acid supplementation focus on prevention of neural tube defects and prescribe that women of childbearing age consume 400 µg folic acid per day from fortified foods, supplements, or both, in addition to consuming food folate from a varied diet [18]. Mandatory food fortification has not yet been introduced in most

European countries [19]. In Europe, the most effective strategy currently is to use a daily folic acid supplement from preconception until the end of the first trimester. However, several studies in the United States suggested that, despite the folic acid fortification implementation in 1998, folic acid intake from fortified foods provides only 100 – 200 µg per day [20, 21]. Thus, also in the United States, most pregnant women are advised to consume a supplement to obtain the recommended amount of folic acid every day [18].

Apart from the preventive effects of folic acid on neural tube defects [2, 3], several lines of research suggested that folates during early pregnancy are important for fetal growth and development, particularly of the brain. Experimental studies in animals showed the negative effects of prenatal folate deficiency on neural development [5] and virtually all inborn errors of folate metabolism are associated with mental retardation [22]. One of the very few studies on human neurodevelopment found no significant association between folate nutritional status of pregnant women and measures of mental and psychomotor development of their children at 5 years of age [11]. The discrepancy between our findings and these results from Tamura et al. may be explained by the small number of mothers with low plasma folate, and the follow-up of a non-population-based sample of educationally and environmentally deprived women [11]. Furthermore, the inconsistency may be explained by differences in measurement of folate status and neurodevelopmental outcome.

Several potential mechanisms may explain the association between folic acid use by pregnant women and behavioral problems in the offspring. First, the higher risk of neural tube defects in children of folate-deficient pregnant women as well as the findings in experimental animal studies indicate direct effects of folates on the developing central nervous system. Many pathways are conceivable to illuminate the mechanism by which folic acid deficiency causes neural tube defects. The question remains whether neural tube defects arise from folate deficiency, or whether folate supplementation prevents neural tube defects by overcoming an intrinsic defect in folate metabolism [1, 23]. Folate is important in neurogenesis, in cell growth and proliferation, and in myelination. Furthermore, folate has been linked to the synthesis of catecholamine neurotransmitters and serotonin [24]. Notwithstanding the uncertainty about the mechanism, the importance for normal brain development of folate intake early in pregnancy is well accepted. Additionally, animal studies provided information on the requirements for folate intake later in fetal gestation. Craciunescu et al. [5] reported that dietary folic acid availability affects mouse brain development long after neural tube closure by influencing cell mitosis and apoptosis in the brain. Our findings may be explained by subtle changes in the developing brain, although we did not find significant mediation by the size of the cerebrum, cerebellum, or ventricles. However, more subtle changes in neuronal and glial growth and proliferation, as well as the effects of folate on the level of neurotransmitters, may give rise to small but significant behavioral disturbances.

Second, indirect effects on fetal growth may explain the effects of prenatal folate deficiency on behavioral problems in young children as well. Iyengar et al. [4] and Scholl et al. [25]

described higher birth weights in children of mothers who used folic acid supplementation during pregnancy. The effects of folate on placental vasculopathy [26] or on DNA methylation of growth hormones in fetal tissues serve as possible mechanisms by which folic acid supplementation affects fetal growth. A lower birth weight has, in turn, been related to behavioral and emotional problems [27]. However, our results do not underscore the hypothesis that birth weight mediates the effects of folate deficiency in prenatal life on behavioral development.

Third, an alternative indirect effect of folate deficiency may be epigenetic dysregulation of genes, whether or not in combination with disease susceptibility genes. Epigenetic modifications are changes in gene expression by DNA methylation and alterations of chromatin structure, rather than entailing a change of DNA sequence [28]. In complex psychiatric disorders like autism and schizophrenia, interactions between susceptibility genes and folate that modify the integrity and or expression of other genes and the metabolism of important cofactors, have been hypothesized as an underlying cause [29, 30].

Fourth, the association between folic acid use during early pregnancy and child behavioral problems may be explained by the epiphenomenona of folic acid use during pregnancy. Our study showed that folic acid supplementation is highly related to socioeconomic class and lifestyle determinants. Furthermore, we showed that maternal characteristics, such as age, educational level, national origin, and psychopathology, are important confounders in the association between folic acid use during pregnancy and child behavior problems. However, these confounding factors did not completely erode the effect of prenatal folates on child behavior. Other maternal health related behaviors, like smoking and alcohol use, could not further explain the association between folic acid use and behavior. Nevertheless, residual confounding remains an issue in all observational studies. For example, intake of other macro- and micronutrients, which were not taken into account in the present study, may well serve as an additional confounding factor.

Apart from residual confounding, some methodological considerations need to be discussed. Selective attrition may have influenced our findings. Children and mothers without information on behavioral outcome differed from families with behavioral information on both the determinant (folic acid use during pregnancy) and several confounding factors. This selective attrition would lead to bias when non-participating women who did not use folic acid supplements, had less behavioral problems. The use of self-reports of folic acid supplement use during pregnancy is a second potential limitation. However, validation studies [31] showed that self-reported intake of folic acid supplements strongly correlated to serum folates. Moreover, we prospectively assessed supplement use in early pregnancy, which makes recall bias unlikely. Another source of possible reporter bias is the use of a mother report to assess behavioral problems. Parents are often the only informants available for children in this very young age. However, maternal perception of problems might lead to misclassification. Future studies that use multiple observers of behavior are needed to clarify this issue. Finally,

the randomized controlled clinical trial is the most powerful instrument to demonstrate the effectiveness of a therapeutic or preventive intervention. However, since the use of folic acid supplements has already been shown to reduce the risk of serious birth defects, it is not ethical to perform a randomized trial to demonstrate the effect of folic acid supplements on behavior. Unfortunately, we were not able to study the effect of folic acid supplement use throughout pregnancy, since we did not assess whether women quit use of folic acid supplements after the completion of the first trimester. The central nervous system further develops and matures in the second and third trimester. It might be interesting to design a clinical trial with randomization of women who quit use of folic acid supplements at 12 weeks of gestation, and women who continue to use folic acid supplements during pregnancy.

In conclusion, folic acid use during early pregnancy prevents child behavioral and emotional problems later in life. The present study underlines the importance of folate supplementation in early pregnancy. Our findings are a step forward in the unraveling of causal mechanisms that link adverse intrauterine environmental factors to mental health. Future studies are needed to replicate the association and to investigate whether prenatal folate deficiency also predicts neurodevelopmental disorders, like attention-deficit hyperactivity disorder and schizophrenia. Despite the current recommendations of adequate folic acid use, still many pregnant women, and in particular those with other vulnerability markers for physical and mental health, do not use folic acid supplements preconceptionally or as soon as pregnancy is known. We recommend development and application of preconception health educational programs to improve intake of folic acid and other health-related behaviors.

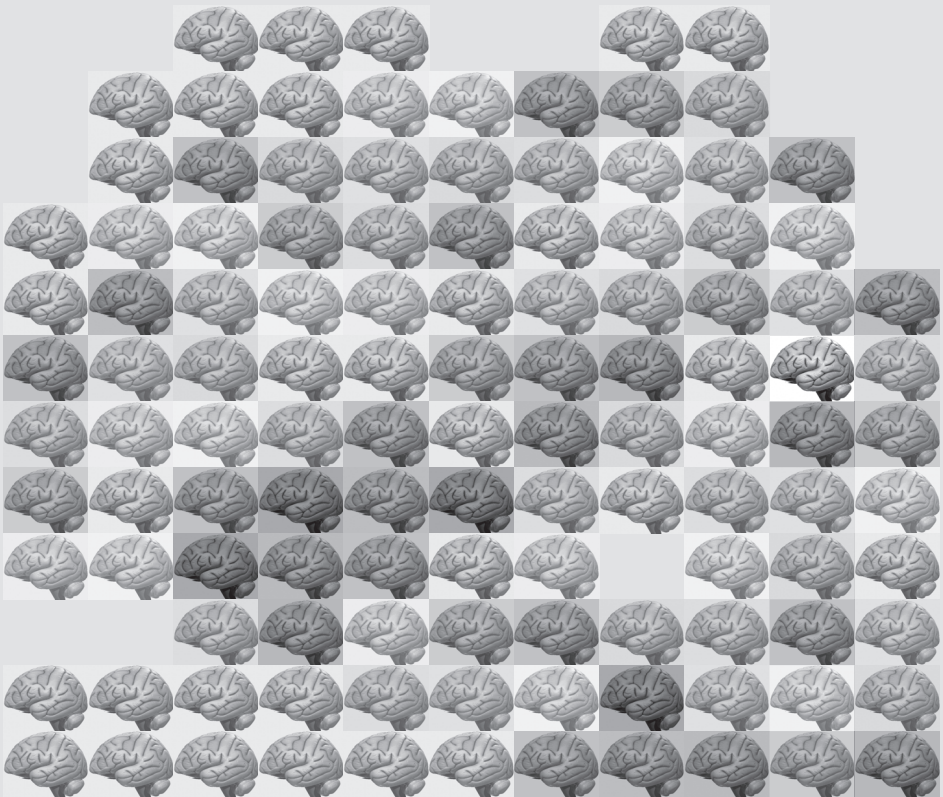
References

1. Bailey, L.B. and J.F. Gregory, 3rd, *Folate metabolism and requirements.* J Nutr, 1999. 129(4): p. 779-82.
2. *Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group.* Lancet, 1991. 338(8760): p. 131-7.
3. Lumley, J., et al., *Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects.* Cochrane Database Syst Rev, 2001(3): p. CD001056.
4. Iyengar, L. and K. Rajalakshmi, *Effect of folic acid supplement on birth weights of infants.* Am J Obstet Gynecol, 1975. 122(3): p. 332-6.
5. Craciunescu, C.N., et al., *Folic acid deficiency during late gestation decreases progenitor cell proliferation and increases apoptosis in fetal mouse brain.* J Nutr, 2004. 134(1): p. 162-6.
6. Xiao, S., et al., *Maternal folate deficiency results in selective upregulation of folate receptors and heterogeneous nuclear ribonucleoprotein-E1 associated with multiple subtle aberrations in fetal tissues.* Birth Defects Res A Clin Mol Teratol, 2005. 73(1): p. 6-28.
7. Zammit, S., et al., *Schizophrenia and neural tube defects: comparisons from an epidemiological perspective.* Schizophr Bull, 2007. 33(4): p. 853-8.
8. Brown, A.S., et al., *Elevated prenatal homocysteine levels as a risk factor for schizophrenia.* Arch Gen Psychiatry, 2007. 64(1): p. 31-9.
9. Erbe, R.E., *Inborn errors of folate metabolism (first of two parts).* N Engl J Med, 1975. 293(15): p. 753-7.
10. Erbe, R.W., *Inborn errors of folate metabolism (second of two parts).* N Engl J Med, 1975. 293(16): p. 807-12.
11. Tamura, T., et al., *Folate status of mothers during pregnancy and mental and psychomotor development of their children at five years of age.* Pediatrics, 2005. 116(3): p. 703-8.
12. Hofman, A., et al., *Growth, development and health from early fetal life until young adulthood: the Generation R Study.* Paediatr Perinat Epidemiol, 2004. 18(1): p. 61-72.
13. Jaddoe, V.W., et al., *The Generation R Study: Design and cohort profile.* Eur J Epidemiol, 2006. 21(6): p. 475-84.
14. Achenbach, T.M. and L.A. Rescorla, *Manual for the ASEBA Preschool Forms & Profiles.* 2000, Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.
15. Tick, N.T., et al., *14-year changes in emotional and behavioral problems of very young Dutch children.* J Am Acad Child Adolesc Psychiatry, 2007. 46(10): p. 1333-40.
16. de Beurs, E., *Brief Symptom Inventory, handleiding.* 2004: Leiden, the Netherlands.
17. Derogatis, L.R. and N. Melisaratos, *The Brief Symptom Inventory: an introductory report.* Psychol Med, 1983. 13(3): p. 595-605.
18. Yang, Q.H., et al., *Race-ethnicity differences in folic acid intake in women of childbearing age in the United States after folic acid fortification: findings from the National Health and Nutrition Examination Survey, 2001-2002.* Am J Clin Nutr, 2007. 85(5): p. 1409-16.
19. Botto, L.D., et al., *Trends of selected malformations in relation to folic acid recommendations and fortification: an international assessment.* Birth Defects Res A Clin Mol Teratol, 2006. 76(10): p. 693-705.
20. Quinlivan, E.P. and J.F. Gregory, 3rd, *Effect of food fortification on folic acid intake in the United States.* Am J Clin Nutr, 2003. 77(1): p. 221-5.
21. Choumenkovitch, S.F., et al., *Folic acid intake from fortification in United States exceeds predictions.* J Nutr, 2002. 132(9): p. 2792-8.
22. Greenblatt, J.M., L.C. Huffman, and A.L. Reiss, *Folic acid in neurodevelopment and child psychiatry.* Prog Neuropsychopharmacol Biol Psychiatry, 1994. 18(4): p. 647-60.
23. Brown, A.S., et al., *Neurobiological plausibility of prenatal nutritional deprivation as a risk factor for schizophrenia.* J Nerv Ment Dis, 1996. 184(2): p. 71-85.

24. Fernstrom, J.D., *Can nutrient supplements modify brain function?* Am J Clin Nutr, 2000. 71(6 Suppl): p. 1669S-75S.
25. Scholl, T.O. and W.G. Johnson, *Folic acid: influence on the outcome of pregnancy.* Am J Clin Nutr, 2000. 71(5 Suppl): p. 1295S-303S.
26. Tamura, T. and M.F. Picciano, *Folate and human reproduction.* Am J Clin Nutr, 2006. 83(5): p. 993-1016.
27. Kelly, Y.J., et al., *Birthweight and behavioural problems in children: a modifiable effect?* Int J Epidemiol, 2001. 30(1): p. 88-94.
28. Muskiet, F.A. and R.F. Kemperman, *Folate and long-chain polyunsaturated fatty acids in psychiatric disease.* J Nutr Biochem, 2006. 17(11): p. 717-27.
29. Johnson, W.G., *DNA polymorphism-diet-cofactor-development hypothesis and the gene-teratogen model for schizophrenia and other developmental disorders.* Am J Med Genet, 1999. 88(4): p. 311-23.
30. Adams, M., et al., *Preliminary evidence for involvement of the folate gene polymorphism 19bp deletion-DHFR in occurrence of autism.* Neurosci Lett, 2007. 422(1): p. 24-9.
31. Brantsaeter, A.L., et al., *Self-reported dietary supplement use is confirmed by biological markers in the Norwegian Mother and Child Cohort Study (MoBa).* Ann Nutr Metab, 2007. 51(2): p. 146-54.

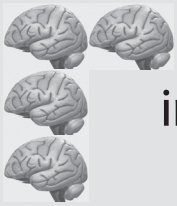
Chapter 3

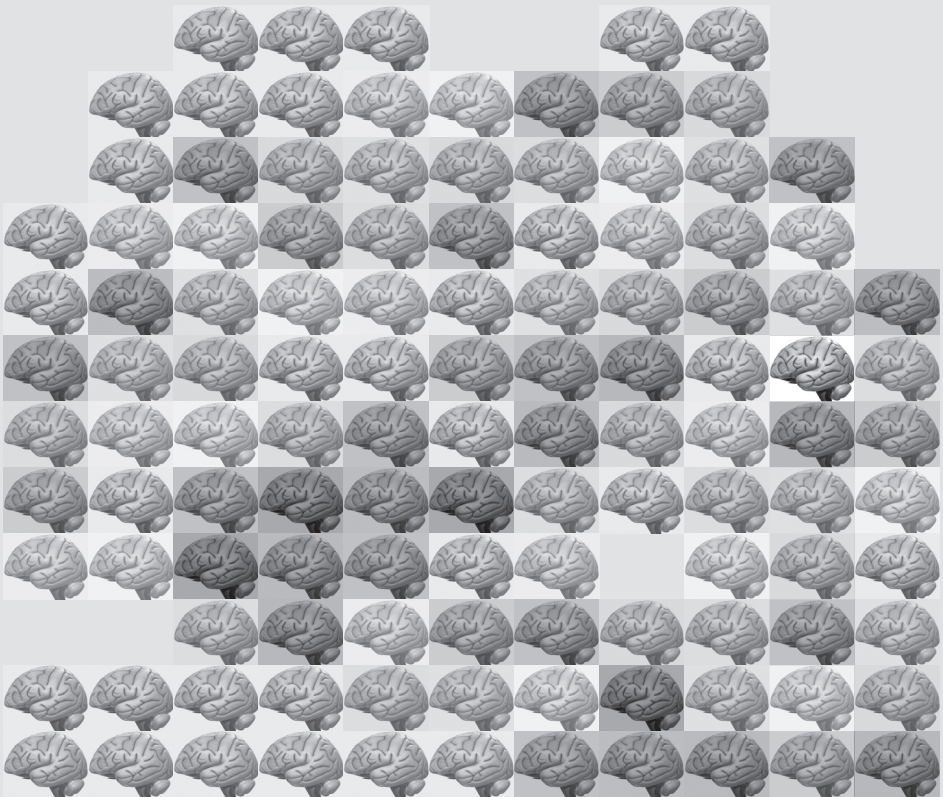
Fetal adaptive mechanisms to an adverse environment



3.1

Intrauterine growth and infant temperamental difficulties





Abstract

Objective: To determine whether intrauterine growth trajectories are associated with temperamental difficulties in infancy.

Method: The Generation R Study is a population-based cohort study from fetal life onwards. Size at different time points during gestation and growth trajectories, calculated on the basis of repeatedly measured fetal growth characteristics, were related to temperamental dimensions, assessed with the Infant Behavior Questionnaire-Revised, in 3,792 infants aged 6 months.

Results: Birth weight, adjusted for gestational age, was negatively associated with activity level and duration of orienting. These associations disappeared after additional adjustment for maternal height, age, educational level, and national origin. Similarly, the negative associations between intrauterine total body weight gain and falling reactivity and activity level diminished after correction for maternal and child characteristics. After full adjustment, reduced fetal weight gain was only related to prolonged duration of orienting. Children scored 0.38 (95% confidence interval: 0.09; 0.68) points higher on duration of orienting per standard deviation decrease in total body weight gain from mid-pregnancy to birth.

Conclusions: After controlling for several genetic and socio-economic status related factors, we found little indication for an association between intrauterine growth trajectories and temperamental difficulties in infants.

Introduction

Evidence from numerous longitudinal studies suggest that part of the vulnerability for mental disorders, like other multifactorial diseases such as coronary heart disease, is shaped in fetal life [1]. Indicators of intrauterine growth, e.g. birth weight or exposure to famine, have been associated with psychiatric disorder in adulthood [2-6]. Furthermore, birth weight has been linked to several emotional and behavioral outcomes in childhood, such as hyperactivity, inattention and depressive behavior [7-12].

Temperament is one aspect of social and emotional behavior that can be measured early in life. Temperament is conceptualized as the constitutionally based individual differences in reactivity and self-regulation [13]. Although there are methodological difficulties when relating temperamental dimensions to children's risk for psychopathology, such as rater biases and overlapping items in instruments measuring temperament and psychopathology [14-16], it is increasingly acknowledged that temperament plays a role in the etiology and maintenance of behavioral problems in childhood and adolescence [13, 17, 18]. Shaw et al. showed that maternal reported negative emotionality and activity level at ages 18 and 24 months were significantly related to externalizing disorders at age 5.5 years [19]. Within the Dunedin longitudinal study, ratings of lack of control, irritability, and distractibility based on the child's behavior during testing sessions at age 3 years predicted externalizing problems at age 9 to 15 years. Early sluggishness predicted later internalizing and externalizing problems for girls, but not for boys [20]. Leve et al. found that child fear/shyness at age 5 years predicted internalizing behavior across a 12-year time span, and that higher impulsivity predicted externalizing behavior for both genders [21].

Theories of temperament and personality have emphasized the constitutional basis of temperament, which is influenced over time by heredity, maturation, and experience. However, only few studies have related indicators of intrauterine growth to infant or child temperament. Studies in pre-term neonates showed that low birth weight infants are more intense in their emotional expression, more distractible, more avoidant or withdrawn, and less regular in their biological rhythms compared to term-born babies [22-24]. In healthy terms, Pesonen et al. found that small body size at birth was related to increased negative affectivity, whereas length at birth was predictive of higher levels of child anger- and sadness-proneness [25].

However, weight, length or thinness at birth are only crude proxies of intrauterine growth and cannot provide information on fetal growth trajectories. Whether the associations between neonatal size and child behavior can be explained by genetically predisposed small size or by growth restriction in utero, is unclear. Animal studies have shown that adverse intrauterine environmental factors, such as maternal malnutrition, maternal stress, and tobacco and alcohol exposure, are related to behavioral development [26-28]. These intrauterine environmental factors are also known to affect intrauterine growth and brain development and can lead to symmetrical or asymmetrical (i.e. a disproportionately large

head compared with body size) growth restriction [29]. Since growth is a cumulative process, and measurement error is relatively large with respect to size in the first half of pregnancy, differences in growth velocity are assessable from the fourth month of gestation onwards [30]. This includes the second and third trimester that form the critical period in which the brain is particularly vulnerable [28].

In the current study, we examined in a large population-based cohort the associations between repeatedly measured fetal growth characteristics and temperamental dimensions in 6-month-old infants. Temperamental continuity is assumed to become apparent after the first few months of infancy [31]. Since we assumed that the influence of postnatal environment accumulates over time and plays a more important role at later ages, we chose to study the effect of intrauterine growth on infant temperament. We hypothesized that reduced growth velocities in the second half of pregnancy were associated to temperamental difficulties at age 6 months. Since size at birth, a crude proxy of intrauterine growth, has been reported to predict both internalizing and externalizing behavior, we explored the effects of growth velocities on negative affectivity, early orienting, and activity level.

Method

Setting

The present study was conducted within the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. The Generation R Study, designed to identify early environmental and genetic determinants of growth, development and health, has been described previously in detail [32, 33]. Enrolment was aimed in early pregnancy. Assessments in pregnancy, including fetal ultrasound examinations and questionnaires, were planned in early pregnancy, mid-pregnancy and late pregnancy. All children were born between April 2002 and January 2006 and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all adult participants.

Study population

In total, 7,654 live born children and their mothers, who were prenatally included, were approached for postnatal consent. Thirty-four children deceased in the first few months. For the present study, we excluded twins (n=155) since growth potentials for individual fetuses in multiple pregnancies are not comparable to singleton pregnancies. The remaining 7,465 chil-

dren were eligible for the present study. Mothers of 928 children did not give full consent for postnatal participation. Due to logistical problems (changed addresses, changed telephone numbers, technical problems with the database), 981 mothers never received the 6-month-questionnaire. Another 1,764 mothers did not complete the 6-month-questionnaire. Information on temperament at age 6 months was available in 3,792 neonates (50.8% of 7,465).

Fetal ultrasound examinations

Fetal ultrasound examinations were carried out by 31 trained sonographers at the visits to the research centers in early, mid- and late pregnancy. The median (95% range) gestational age for the fetal ultrasound examinations in early, mid- and late pregnancy was 12.9 (10.6 – 17.3) weeks, 20.5 (18.6 – 23.0) weeks and 30.4 (28.5 – 32.8) weeks. Measurements used for the present study included head circumference, abdominal circumference, and femur length and were done using standardized techniques. Estimated fetal weight was calculated by Hadlock's formula using head circumference, abdominal circumference, and femur length [34]. In line with the reproducibility for growth parameters throughout pregnancy reported by Perni et al.[35], the intra- and inter-observer reliability of fetal biometry measurements in early pregnancy within the Generation R Study is high. For reliability analyses, two raters each measured all growth parameters in 21 pregnant women twice. Intra-observer intraclass correlation coefficients varied from 0.985 for abdominal circumference to 0.995 for biparietal diameter and head circumference, inter-observer intraclass correlation coefficients varied from 0.980 for abdominal circumference to 0.994 for biparietal diameter, with coefficients of variation between 1.8 and 3.8%.

Infant temperament

Approximately 6 months after delivery, child temperament was assessed using an adapted version of six scales of the Infant Behavior Questionnaire – Revised (IBQ-R) [36]. The IBQ-R asks mothers to rate the frequency of specific behaviors observed over the past week. We chose six out of 14 scales, since we judged these scales of particular importance for the later prediction of the most prevalent behavioral problems in children such as anxiety, aggressive behavior, and attention problems. The complete original instrument of 191 items was not feasible, since long and time-consuming questionnaires in a multidisciplinary study with numerous assessments could increase the risk of attrition. We did not perform a formal validation study, but carried out a pilot study. As a result of this pilot study, a few items per scale were dropped since mothers judged items as overlapping, probably due to wording of the translated version. The remaining 10 - 15 items per scale loaded on the same temperamental dimensions as in the original instrument. The six scales in our adapted version included Activity Level, Distress to Limitations, Fear, Duration of Orienting, Falling reactivity, and Sadness.

Activity level relates to gross motor activity and squirming. Distress to limitations refers to negative emotionality and reaction to frustrating situations. Fear includes rejection of new objects and persons. Duration of orienting comprises items on attention and distractibility. Falling reactivity refers to rate of recovery from peak distress or frustration. Finally, questions about sadness relate to lowered mood due to personal suffering or object loss. We adapted the original 7-point scale to a 3-point scale (0=never present, 1=sometimes present and 2=often present) because respondents in the pilot study seldom use the extreme positions of scales with more than 5 categories. Moreover, when a large number of individual items are designed to be summed to create a scale score, it has been suggested that reducing the number of levels to three will not result in significant loss of information [37]. Higher scores on the scales, besides on falling reactivity, indicate more difficult behavior. The scores for each scale were calculated by dividing the sum of the items by the number of completed items. Internal consistencies for the adapted IBQ-R ranged from 0.70 for duration of orienting to 0.85 for fear, which is comparable to the internal consistencies of the original IBQ-R [36].

Covariates

In line with reports from the ALSPAC cohort on fetal growth and childhood behavior [11], we considered socio-economic status related variables (maternal educational level, maternal smoking in pregnancy, national origin), obstetric and neonatal variables (gestational age at birth, parity) and other known determinants of mother' reported infant temperament (infant gender, infant age, maternal anxiety, maternal depression) as possible confounders. Additionally, we adjusted our associations for both maternal and paternal height to control for possible genetic confounding [38], and for several perinatal and obstetric complications (maternal hypertension, pre-eclampsia, gestational diabetes, Apgar scores after 1 and 5 minutes and mode of delivery) since these factors have been shown to predict infant temperament in preterm born infants [24]. Date of birth, birth weight, gender, and perinatal and obstetric complications were obtained from midwife and hospital registries at birth. We established gestational age by using the fetal ultrasound examinations within the Generation R Study. Height of the parents was measured at their first visit to the research center. The first questionnaire provided information on maternal educational level, country of birth of the parents, and parity. The participating child was of non-Dutch origin if one of the parents was born abroad. When both parents were born abroad, we used the mother's country of birth to determine the child's origin. Maternal smoking in pregnancy was assessed in early, mid- and late pregnancy by questionnaire and categorized into 'no smoking', 'smoking until pregnancy was known' and 'continued smoking during pregnancy'. For assessment of maternal anxiety and depression in mid-pregnancy, we used two scales of the Brief Symptom Inventory (BSI), a validated self-report questionnaire with 53 items scored on a 5-point scale [39, 40]. Midwife and hospital registries at birth yielded information on mode of delivery, Apgar scores and

umbilical artery cord blood analyses. Mode of delivery was classified as 'caesarean section', 'spontaneous vaginal delivery' and 'instrumental vaginal delivery'.

Statistical analyses

To examine whether non-response was selective, we compared gestational duration, birth weight, age of mother, maternal educational level, maternal smoking during pregnancy, and national origin of children with information on fetal growth and temperament to eligible children in the postnatal phase without information on temperament. Non-responders were defined as the mothers of singleton live births with valid information on fetal growth who refused to give consent for postnatal questionnaires, never received the 6-month-questionnaire, or did not complete the 6-month-questionnaire. The core variables of the mothers who did not respond to the questionnaire as well as those of mothers who did not receive the questionnaire were compared to core variables of participating mothers.

Next, we examined the associations of fetal estimated weight in mid- and late pregnancy and birth weight with the six scales of temperament, using multivariable linear regression. As nonlinear associations between fetal size characteristics and infant alertness have been reported, we tested the improvement of model fit with additional inclusion of quadratic terms of the fetal estimated weight. Significant improvement of the fit of a model with a quadratic term signifies that the association is not linear, but curvilinear, which indicate that there is an optimal body size with respect to a particular outcome.

Second, to test our main hypothesis, we analyzed the associations between repeatedly measured growth characteristics and temperamental scales using latent growth curve modeling. Latent growth curve models consider change over time through underlying latent growth parameters (e.g. intercept and linear slope), and captures individual variations around these growth parameters as random effects. One of the key advantages of latent growth curve models is the ability to use all available data. Under the assumption that missing ultrasound measurements were random (i.e. not dependent on the unrecorded values of growth), these growth curve models were shown to give valid results [41]. The models include values of growth characteristics of individuals who had less than three ultrasounds, which is a better approach than excluding these individuals in a complete case analysis. However, we did not impute the missing values on temperament, which are likely to be non-random. Since the association between fetal growth and gestational age is not linear [42], we transformed the fetal growth characteristics to standard deviation scores, which are linearly related to gestational age. These standard deviation scores were constructed using reference growth curves from the total study population of Generation R [42]. From the repeated measures of standard deviation scores of fetal growth, we estimated two continuous latent variables corresponding to the two components of growth (intercept; initial level, and linear slope; growth with age). The time intervals between repeated ultrasound measurements were fixed

at group level. All six temperamental dimensions were included in one model and regressed on the growth parameters. Model fit was determined through the Comparative Fit Index (CFI) [43]; critical value ≥ .95, and the Root Mean Square Error of Approximation (RMSEA) [44]; critical value ≤ .08. All associations were controlled for age and gender of the child. The other covariates were selected as a result of exploratory analyses, and were included in the analyses if significantly related (p< .05) with both fetal growth and with three or more temperamental dimensions. The associations between fetal growth and the six temperamental scales were fitted in one, multivariate, model. We additionally carried out a post-hoc Bonferroni adjustment to correct for the three hypotheses with regard to different fetal growth characteristics (total body weight, head circumference, and abdominal circumference). Measures of association are presented with their 95% confidence intervals (CI). Statistical analyses were carried out using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA) and Mplus version 4.2 [45] for the latent growth curve analyses.

Results

Table 1 presents general characteristics of participating children. Of this sample, 49.8% was male. Birth weight was larger in boys than in girls (t=8.14, p-value < 0.001). Gestational duration was slightly longer in boys compared to girls in our study (Mann-Whitney Z=-2.16, p-value = 0.03). In the total group, gestational duration ranged from 29.9 to 43.4 weeks, with a median of 40.1 weeks. Birth weight ranged from 930 to 5260 grams, with a mean of 3465 grams.

Analyses of missing temperamental data showed that children without information on temperament at age 6 months had on average 69 (95% confidence interval (CI): 43; 95) grams lower birth weight, and were less often of Dutch origin (48.4% vs. 65.3%, χ^2=273, df = 7, p-value < .001). Their mothers were 2.1 (95% CI: 1.8; 2.3) years younger, lower educated (% primary education 15.7% vs. 5.4%, χ^2=367, df = 2, p-value < .001), and more often continued to smoke during pregnancy (19.8% vs. 12.7%, χ^2=63, df = 2, p-value < .001). Mothers who did not receive questionnaires and mother who did not respond to the 6-month-questionnaire differed from participating mothers in gestational age at birth, birth weight, maternal age, maternal educational level, national origin and maternal smoking during pregnancy.

Table 2 presents the associations of total body weight with temperamental dimensions at age 6 months. Body weight was measured in mid-pregnancy, in late pregnancy, and at birth. The total scores per temperamental dimension ranged from 0 to 2. First, we report the crude association between gestational age-adjusted standard deviation scores of total body weight and infant temperamental dimensions. In these unadjusted analyses, weight at birth was negatively associated to infant activity level and prolonged duration of orienting. However, the effects were small: Children scored 0.03 points higher on activity level and 0.02 points

Table 1. Subject characteristics

	Boys n = 1,881	Girls N = 1,911
Child characteristics		
Gestational duration (weeks)	40.3 (36.0 – 42.4)	40.1 (36.3 – 42.3)
Birth weight (grams)	3532 (545)	3385 (528)
National origin (%)		
Dutch	66.3	63.8
Turkish	5.6	5.9
Moroccan	3.7	3.9
Cape Verdean	1.5	1.4
Dutch Antillean	1.8	3.4
Surinamese	5.8	5.4
Other non-Western	6.0	6.4
Other Western	9.3	9.8
Maternal characteristics		
Maternal age (years)	31.0 (4.8)	31.0 (4.6)
Maternal height (cm)	168.7 (7.1)	168.3 (7.4)
Paternal height (cm)	182.8 (7.7)	182.8 (7.5)
Maternal educational level (%)		
Primary	5.5	5.8
Secondary	38.7	37.9
High	55.8	56.4
Parity (% nulliparous)	60.3	59.9
Maternal smoking in pregnancy(%)		
No	78.2	79.5
Quit in early pregnancy	8.0	8.6
Continued during pregnancy	13.8	11.9
Mode of delivery (%)		
Spontaneous vaginal	66.7	69.8
Instrumental vaginal	19.8	18.0
Caesarean section	13.5	12.3

Values are means (SD) for continuous normally distributed variables, medians (95% range) for continuous non-normally distributed variables (gestational age) and percentages for categorical variables.

higher on prolonged duration of orienting to single objects per standard deviation smaller total body weight. Next, we adjusted the associations for all covariates to show the effect of confounding variables. Correction for national origin, maternal educational level, maternal age and height eliminated the effects of total body weight at birth on these temperamental dimensions (fully adjusted models). In mid- and late pregnancy (gestational age ranging from 18-23 weeks and 29-33 weeks, respectively) total body weight was not associated to temperamental dimensions. Inclusion of quadratic terms of the estimated fetal weight or birth weight in the models with temperamental dimensions did not significantly improve the models' fit.

Next, we examined whether fetal growth trajectories were related to temperament in infancy (table 3). We constructed a model in which all six temperamental scales were regressed

Table 2. Associations between fetal size and infant temperament

	Activity level	Distress to limitations	Fear	Duration of orienting	Falling reactivity†	Sadness
Estimated fetal weight in mid-pregnancy (SD)						
I. Unadjusted model	-0.00 (-0.01; 0.01)	-0.00 (-0.01; 0.01)	0.01 (-0.00; 0.02)	-0.01 (-0.03; 0.00)	0.00 (-0.01; 0.01)	-0.00 (-0.01; 0.01)
II. Fully adjusted model	-0.00 (-0.01; 0.01)	-0.00 (-0.01; 0.01)	0.01 (-0.00; 0.02)	-0.01 (-0.02; 0.00)	0.00 (-0.01; 0.01)	-0.00 (-0.01; 0.01)
Estimated fetal weight in late pregnancy (SD)						
I. Unadjusted model	-0.01 (-0.02; 0.00)	-0.00 (-0.01; 0.01)	-0.00 (-0.01; 0.01)	-0.01 (-0.02; 0.00)*	0.01 (0.00; 0.02)*	-0.00 (-0.01; 0.01)
II. Fully adjusted model	0.00 (-0.01; 0.01)	0.01 (-0.00; 0.02)	0.01 (-0.00; 0.02)	-0.01 (-0.02; 0.01)	0.01 (-0.01; 0.02)	0.00 (-0.01; 0.01)
Birth weight (SD)						
I. Unadjusted model	-0.03 (-0.04;-0.02)**	-0.01 (-0.02; 0.01)	-0.01 (-0.02; 0.00)	-0.02 (-0.04;-0.02)**	0.01 (0.00; 0.02)*	0.00 (-0.01; 0.01)
II. Fully adjusted model	-0.01 (-0.02; 0.00)	0.01 (-0.00; 0.02)	0.01 (-0.00; 0.02)	-0.02 (-0.03; 0.01)	0.00 (-0.01; 0.01)	0.00 (-0.01; 0.01)

Values are regression coefficients from multivariable linear regression and reflect the difference in temperamental score per standard deviation of estimated fetal weight or birth weight. The standard deviation scores for total body weight were constructed using growth reference curves and are adjusted for gestational age. We present the unadjusted and the fully adjusted models to show the effect of confounding variables. Fully adjusted models were adjusted for age, gender and national origin of the child, as well as for maternal age, maternal height, maternal educational level, maternal smoking during pregnancy, and maternal anxiety during pregnancy. † Higher scores on falling reactivity are interpreted as less difficult behavior, whereas higher scores on the other scales indicate that the infant displays more difficult behavior. * p< 0.05 **p<0.01. Associations that remained significant after Bonferroni adjustment are presented in bold.

on the latent growth parameters of standard deviation scores of total body weight. This model had good to acceptable fit: Comparative Fit Index (CFI)=0.98, root mean squared error of approximation (RMSEA) = 0.08. In the unadjusted model, the slope of total body weight gain during pregnancy was negatively associated with activity level and duration of orienting. Furthermore, the slope was positively associated with falling reactivity, a scale where, in contract to the others, a higher score indicates more easy temperament. Children scored 0.62 points higher on activity and 0.53 points higher on prolonged duration of orienting per standard deviation decrease in total body weight from mid-pregnancy to birth. Children scored 0.28 points lower on falling reactivity per standard deviation decrease in fetal weight. Then, we adjusted our models for the covariates that were both related to three or more temperamental scales as well as to fetal growth. The fit indices for the adjusted models were CFI=0.98 and RMSEA = 0.05. National origin, maternal age, maternal length, and maternal educational level diminished the effects of intrauterine growth on several temperamental dimensions. The effects of the slope of total body weight gain both on activity level and on falling reactivity were no longer significant after correction for these factors. Only the effect of reduced fetal growth on prolonged duration of orienting remained significant after full adjustment for all confounders and for multiple hypothesis testing. Per standard deviation decrease in total body weight gain from mid-pregnancy to birth, children scored 0.38 points higher on duration of orienting. The positive association between slope of total body weight gain and distress to limitations, as well as the intercept effects on fear and duration of orienting did not reach significance level after correction for multiple hypotheses testing. Next to the effects of growth of the total body of the fetus, we examined the effects of growth of fetal

Table 3. Associations between fetal growth and infant temperament

	Activity level	Distress to limitations	Fear	Duration of orienting	Falling reactivity†	Sadness
Growth parameters for fetal weight gain						
I. Unadjusted model						
Intercept	-0.02 (-0.04; -0.00)*	-0.00 (-0.02; 0.01)	0.00 (-0.01; 0.02)	-0.03 (-0.05;-0.01)*	0.01 (-0.00; 0.03)	-0.00 (-0.02; 0.01)
Slope	-0.62 (-0.89;-0.35)**	-0.08 (-0.34; 0.17)	-0.32 (-0.56;-0.07)*	-0.53 (-0.82;-0.24)**	0.28 (0.04; 0.51)**	0.08 (-0.16; 0.31)
II. Fully adjusted model						
Intercept	-0.00 (-0.02; 0.01)	0.01 (-0.01; 0.03)	0.02 (0.00; 0.03)*	-0.02 (-0.04;-0.01)*	0.00 (-0.01; 0.02)	0.00 (-0.01; 0.01)
Slope	-0.15 (-0.40; 0.11)	0.26 (0.01; 0.51)*	0.08 (-0.16; 0.32)	-0.38 (-0.68;-0.09)**	0.05 (-0.18; 0.29)	0.16 (-0.08; 0.39)

Values are regression coefficients from latent growth curve analyses and reflect the difference in temperamental score per standard deviation intercept (in mid-pregnancy) or growth from mid-pregnancy to birth. Gestational age adjusted standard deviation scores for total body weight were constructed using growth reference curves. We present the unadjusted and the fully adjusted models to show the effect of confounding variables.
Fully adjusted models were adjusted for age, gender, and national origin of the child, and maternal educational level, maternal age, maternal height, maternal smoking during pregnancy, and maternal anxiety during pregnancy.
† Higher scores on falling reactivity are interpreted as less difficult behavior, whereas higher scores on the other scales indicate that the infant displays more difficult behavior.
* p< 0.05 **p<0.01. Associations that remained significant after Bonferroni adjustment are presented in bold.
Fit indices for the unadjusted model were CFI=0.98 and RMSEA = 0.08,
and for the fully adjusted model: CFI=0.98 and RMSEA = 0.05.

head circumference and growth of fetal abdominal circumference from early to late pregnancy. None of the intercepts or slopes of these fetal characteristics was significantly related to infant temperamental dimensions after adjustment for all covariates (data not shown).

Discussion

In a population-based study of 3,792 infants, we found little indication for an association between intrauterine growth and temperamental difficulties. The negative association between birth weight and infant temperament was spurious and disappeared when national origin, maternal educational level, maternal age, and maternal height were controlled for. Full adjustment for confounding variables diminished the effects of fetal growth trajectories on most temperamental dimensions as well. Reduced gain of total body weight from mid-pregnancy to birth was only related to prolonged duration of orienting to single objects.

Our findings contradict earlier studies that reported associations between birth weight and temperamental factors [22, 23]. Moreover, our results are generally not in line with findings in large studies in community-dwelling subjects, which found negative associations between birth weight and behavioral and emotional problems in childhood [11, 12]. We will discuss several possible explanations for these discrepancies.

First, some of the populations studied in relation to temperamental factors were not alike with respect to severity of growth retardation and prematurity. The studies of Weiss et al. [22] and Hughes et al. [23] reported higher scores on arrhythmic biorhythms, withdrawing, slow

adaptability, negative mood, and distractibility in low birth weight preterms compared to the standardized normative means for full-term infants. However, variations in birth weight or gestational age within the sample did not predict any of the temperamental outcomes [22]. The studies that related size at birth to behavioral outcomes were not similar to our population with respect to age. Moreover, we should be cautious in interpreting temperamental factors as stable throughout development and predictive for behavioral and emotional problems in later childhood [14, 46]. There are methodological difficulties in relating temperament to behavioral development, such as rater biases and overlapping items in instruments measuring temperament and psychopathology [14-16]. However, several studies found that temperamental dimensions such as activity level, negative affectivity, and early orienting predict phenotypic expressions of childhood-onset behavioral problems [47-51].

Secondly, in studies associating birth weight to temperamental factors, as well as in those associating size at birth to behavioral problems, control for confounding differed from our strategy. Pesonen et al. [52] found a negative effect of low birth weight on fearfulness and negative reactivity in dichotomized analyses that compared children in the lowest 10th percentile of birth weight to the other children. They adjusted only for gender of the children. In an extended sample (n=416) from the same study, Pesonen et al. [25] additionally adjusted for length of gestation, maternal body mass index, age, maternal education, and maternal smoking status. Lower birth weight was only related to greater negative affectivity among children born during the early term weeks of gestation. Kelly et al. [12] described that maternal smoking during pregnancy, maternal age, and maternal psychological status attenuated the associations between birth weight and hyperactivity and peer problems. Wiles et al. [11] found that the association between birth weight and total difficulties at age 81 months disappeared after adjustment for birth length, and for maternal smoking, maternal age, parity, socioeconomic class, body mass index, and maternal depression / anxiety. Obel et al. [38] suggested earlier that there might be a common genetic link between height and behavioral problems, which confound the association between fetal growth restriction and behavioral problems. Indeed, our additional adjustment for maternal length and national origin explained both the associations between birth weight and temperament, and the associations between reduced intrauterine growth and temperamental dimensions. On the other hand, we may have overcontrolled our analyses by adjusting for factors possibly preceding fetal growth in the causal pathway to infant temperament. Maternal smoking and maternal stress during pregnancy are known factors that adversely affect fetal growth. However, distinguishing the effects of epiphenomena of smoking (i.e. poor prenatal care, dietary restriction, low socioeconomic status) from the true etiologic role for prenatal cigarette exposure in reduced fetal growth and impaired brain development remains a challenge [53]. Regarding gestational stress, one might argue that the increased risk of difficult temperament or behavioral disorders in offspring of mothers who were anxious during pregnancy may not be entirely mediated by prenatal risk effects but simply index an underlying inherited liability to anxiety

[54]. The literature on fetal programming still debates whether the association between size at birth and adult disease can be explained by alterations in fetal nutrition and endocrine status, or by genetic factors, which influence both fetal growth and predisposition for adult disease [55]. The most important confounding variables in our analyses were national origin, maternal age, and maternal height. These factors may represent both physiological and pathological effects on fetal growth. For example, national origin and maternal height may reflect constitutional growth potential [56], but differences in ethnic background or maternal size might also reflect differences in feeding habits throughout the mother's life or other lifestyle and socio-economic status related factors [57, 58].

Some methodological limitations of the present study need to be discussed. First, selection effects may have influenced our findings. Our analyses on missing data indicate that attrition, both of mothers who did not receive questionnaires and of mothers who did not respond, was not random. There was a selective dropout of children from younger, lower educated mothers of non-Dutch origin. These children are of increased risk for intrauterine growth restriction [59] as well as for temperamental difficulties [60]. This type of attrition might introduce bias when the association between growth trajectories and infant temperament was significantly larger in non-participating children than in participating children. Due to these selection effects, our results may not be representative of the total population of infants in Rotterdam. Second, using a mother-report measure to assess infant temperament might introduce reporter bias, since these questionnaires reflect not only a report of actual child behavior but also contain a component colored by parental characteristics [46]. However, since mothers were blind to their infant's growth trajectory, we assume this type of misclassification does not depend on the determinant. Furthermore, maternal report of temperament takes advantage of the primary caregivers' opportunity to observe her infant across different contexts [36]. Third, we need to mention the limited categories in our adapted version of the IBQ, which may have reduced the variation of temperamental scores. This would decrease the chance of detecting statistically significant effects.

In conclusion, we found only little indication that intrauterine growth trajectories are associated with infant temperament. Our findings imply that the associations between body size at birth and behavior in infancy are explained by factors influencing both fetal growth and vulnerability for temperamental difficulties. Future research into the associations between indicators of intrauterine growth and behavioral and emotional disorders should focus on both environmental and genetic influences.

References

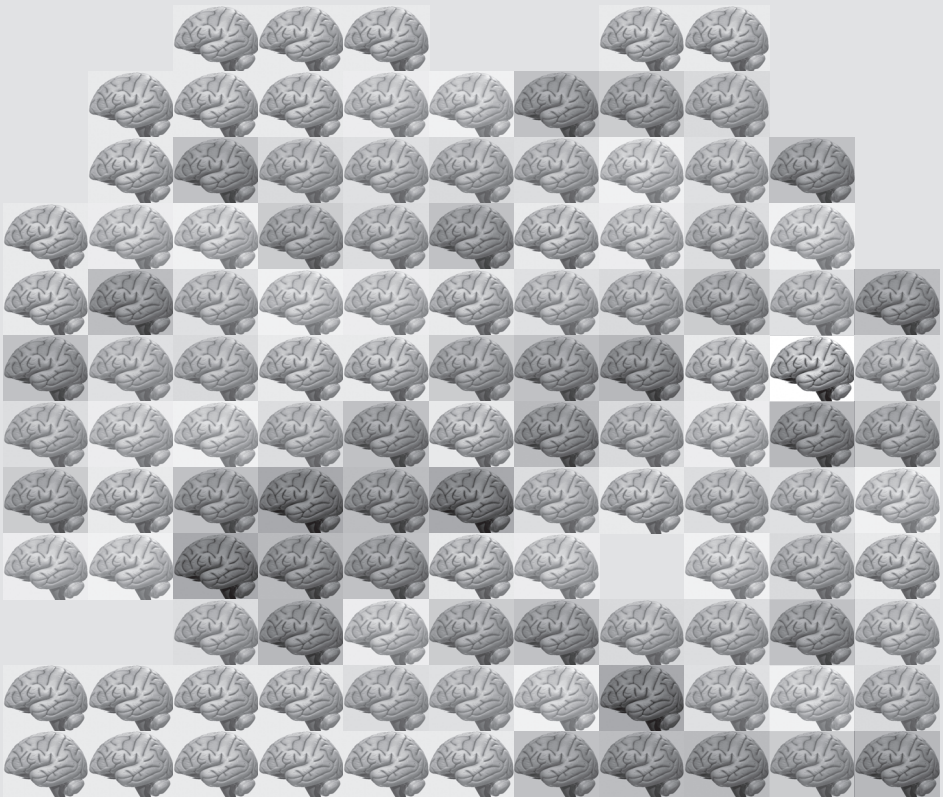
1. Barker, D.J., *Fetal origins of coronary heart disease.* BMJ, 1995. 311(6998): p. 171-4.
2. Wahlbeck, K., et al., *Association of schizophrenia with low maternal body mass index, small size at birth, and thinness during childhood.* Arch Gen Psychiatry, 2001. 58(1): p. 48-52.
3. Gale, C.R. and C.N. Martyn, *Birth weight and later risk of depression in a national birth cohort.* Br J Psychiatry, 2004. 184: p. 28-33.
4. Cannon, M., P.B. Jones, and R.M. Murray, *Obstetric complications and schizophrenia: historical and meta-analytic review.* Am J Psychiatry, 2002. 159(7): p. 1080-92.
5. Neugebauer, R., H.W. Hoek, and E. Susser, *Prenatal exposure to wartime famine and development of antisocial personality disorder in early adulthood.* JAMA, 1999. 282(5): p. 455-62.
6. Susser, E., et al., *Schizophrenia after prenatal famine. Further evidence.* Arch Gen Psychiatry, 1996. 53(1): p. 25-31.
7. Rice, F., G.T. Harold, and A. Thapar, *The effect of birth-weight with genetic susceptibility on depressive symptoms in childhood and adolescence.* Eur Child Adolesc Psychiatry, 2006. 15(7): p. 383-91.
8. Lahti, J., et al., *Small body size at birth and behavioural symptoms of ADHD in children aged five to six years.* J Child Psychol Psychiatry, 2006. 47(11): p. 1167-74.
9. Gunther, N., et al., *Association of mental health problems in childhood with prenatal and postnatal physical growth.* Eur Psychiatry, 2005. 20(3): p. 277-86.
10. van Os, J., et al., *A prospective twin study of birth weight discordance and child problem behavior.* Biol Psychiatry, 2001. 50(8): p. 593-9.
11. Wiles, N.J., et al., *Fetal growth and childhood behavioral problems: results from the ALSPAC cohort.* Am J Epidemiol, 2006. 163(9): p. 829-37.
12. Kelly, Y.J., et al., *Birthweight and behavioural problems in children: a modifiable effect?* Int J Epidemiol, 2001. 30(1): p. 88-94.
13. Rothbart, M.K. and M.I. Posner, *Temperament, attention, and developmental psychopathology*, in *Developmental Psychopathology (2nd ed.).: Developmental Neuroscience*, D. Cicchetti and D. Cohen, Editors. 2006, Wiley: New York. p. 465 - 501.
14. Lahey, B.B., *Commentary: role of temperament in developmental models of psychopathology.* J Clin Child Adolesc Psychol, 2004. 33(1): p. 88-93.
15. Rothbart, M.K., *Commentary: differentiated measures of temperament and multiple pathways to childhood disorders.* J Clin Child Adolesc Psychol, 2004. 33(1): p. 82-7.
16. Frick, P.J., *Integrating research on temperament and childhood psychopathology: its pitfalls and promise.* J Clin Child Adolesc Psychol, 2004. 33(1): p. 2-7.
17. Nigg, J.T., *Temperament and developmental psychopathology.* J Child Psychol Psychiatry, 2006. 47(3-4): p. 395-422.
18. Muris, P. and T.H. Ollendick, *The role of temperament in the etiology of child psychopathology.* Clin Child Fam Psychol Rev, 2005. 8(4): p. 271-89.
19. Shaw, D.S., et al., *Infant and toddler pathways leading to early externalizing disorders.* J Am Acad Child Adolesc Psychiatry, 2001. 40(1): p. 36-43.
20. Caspi, A., et al., *Temperamental origins of child and adolescent behavior problems: from age three to age fifteen.* Child Dev, 1995. 66(1): p. 55-68.
21. Leve, L.D., H.K. Kim, and K.C. Pears, *Childhood temperament and family environment as predictors of internalizing and externalizing trajectories from ages 5 to 17.* J Abnorm Child Psychol, 2005. 33(5): p. 505-20.
22. Weiss, S.J., M.S. Jonn-Seed, and P. Wilson, *The temperament of pre-term, low birth weight infants and its potential biological substrates.* Res Nurs Health, 2004. 27(6): p. 392-402.
23. Hughes, M.B., et al., *Temperament characteristics of premature infants in the first year of life.* J Dev Behav Pediatr, 2002. 23(6): p. 430-5.

24. Larroque, B., et al., *Temperament at 9 months of very preterm infants born at less than 29 weeks' gestation: the Epipage study.* J Dev Behav Pediatr, 2005. 26(1): p. 48-55.
25. Pesonen, A.K., et al., *Fetal programming of temperamental negative affectivity among children born healthy at term.* Dev Psychobiol, 2006. 48(8): p. 633-43.
26. Dickerson, P.A., et al., *Early emergence of increased fearful behavior in prenatally stressed rats.* Physiol Behav, 2005. 86(4): p. 586-93.
27. Wadhwa, P.D., *Psychoneuroendocrine processes in human pregnancy influence fetal development and health.* Psychoneuroendocrinology, 2005. 30(8): p. 724-43.
28. Li, Y. and H. Wang, *In utero exposure to tobacco and alcohol modifies neurobehavioral development in mice offspring: consideration a role of oxidative stress.* Pharmacol Res, 2004. 49(5): p. 467-73.
29. Lin, C.C. and J. Santolaya-Forgas, *Current concepts of fetal growth restriction: part I. Causes, classification, and pathophysiology.* Obstet Gynecol, 1998. 92(6): p. 1044-55.
30. Milani, S., et al., *Differences in size at birth are determined by differences in growth velocity during early prenatal life.* Pediatr Res, 2005. 57(2): p. 205-10.
31. Komsi, N., et al., *Continuity of temperament from infancy to middle childhood.* Infant Behav Dev, 2006. 29(4): p. 494-508.
32. Jaddoe, V.W., et al., *The Generation R Study: Design and cohort profile.* Eur J Epidemiol, 2006. 21(6): p. 475-84.
33. Hofman, A., et al., *Growth, development and health from early fetal life until young adulthood: the Generation R Study.* Paediatr Perinat Epidemiol, 2004. 18(1): p. 61-72.
34. Hadlock, F.P., et al., *Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements.* Radiology, 1984. 150(2): p. 535-40.
35. Perni, S.C., et al., *Intraobserver and interobserver reproducibility of fetal biometry.* Ultrasound Obstet Gynecol, 2004. 24(6): p. 654-8.
36. Gartstein, M.A. and M.K. Rothbart, *Studying infant temperament via the Revised Infant Behavior Questionnaire.* Infant Behavior & Development, 2003. 26: p. 64 - 86.
37. Streiner, D.L. and G.R. Norman, *Health Measurement Scales: a practical guide to their development and use*. 3rd ed. 2003, New York: Oxford University Press.
38. Obel, C., T.B. Henriksen, and J. Olsen, *Re: "Fetal growth and childhood behavioral problems: Results from the ALSPAC cohort".* Am J Epidemiol, 2006. 164(9): p. 916-7; author reply 917.
39. Derogatis, L.R. and N. Melisaratos, *The Brief Symptom Inventory: an introductory report.* Psychol Med, 1983. 13(3): p. 595-605.
40. de Beurs, E., *Brief Symptom Inventory, handleiding.* 2004: Leiden, the Netherlands.
41. Llabre, M.M., et al., *Applying latent growth curve modeling to the investigation of individual differences in cardiovascular recovery from stress.* Psychosom Med, 2004. 66(1): p. 29-41.
42. Verburg, B.O., et al., *New charts for ultrasound dating of pregnancy and assessment of fetal growth, longitudinal data from a population-based cohort study.* Ultrasound Obstet Gynecol, 2007. in press.
43. Bentler, P.M., *Comparative fit indexes in structural models.* Psychol Bull, 1990. 107(2): p. 238-46.
44. Brown, M.W. and R. Cudeck, *Alternative ways of assessing model fit.*, in *Testing structural equation models*, K.A. Bollen and J.S. Long, Editors. 1993, Sage: Newbury Park, CA. p. 136 - 162.
45. Muthén, L.K. and B.O. Muthén, *Mplus. Statistical analyses with latent variables. User's guide (4.2 edn).* 1998 - 2006, Los Angeles: Muthén & Muthén.
46. Seifer, R., et al., *Infant temperament measured by multiple observations and mother report.* Child Dev, 1994. 65(5): p. 1478-90.
47. Nigg, J.T., H.H. Goldsmith, and J. Sachek, *Temperament and attention deficit hyperactivity disorder: the development of a multiple pathway model.* J Clin Child Adolesc Psychol, 2004. 33(1): p. 42-53.
48. Colombo, J., *The development of visual attention in infancy.* Annu Rev Psychol, 2001. 52: p. 337-67.
49. Colombo, J., et al., *Individual differences in infant visual attention: are short lookers faster processors or feature processors?* Child Dev, 1991. 62(6): p. 1247-57.

50. Zwaigenbaum, L., et al., *Behavioral manifestations of autism in the first year of life.* Int J Dev Neurosci, 2005. 23(2-3): p. 143-52.
51. Fox, N.A., et al., *Behavioral inhibition: linking biology and behavior within a developmental framework.* Annu Rev Psychol, 2005. 56: p. 235-62.
52. Pesonen, A.K., et al., *Do gestational age and weight for gestational age predict concordance in parental perceptions of infant temperament?* J Pediatr Psychol, 2006. 31(3): p. 331-6.
53. Ernst, M., E.T. Moolchan, and M.L. Robinson, *Behavioral and neural consequences of prenatal exposure to nicotine.* J Am Acad Child Adolesc Psychiatry, 2001. 40(6): p. 630-41.
54. Thapar, A., et al., *Do intrauterine or genetic influences explain the foetal origins of chronic disease? A novel experimental method for disentangling effects.* BMC Med Res Methodol, 2007. 7: p. 25.
55. Lunde, A., et al., *Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data.* Am J Epidemiol, 2007. 165(7): p. 734-41.
56. Zhang, X., et al., *Are babies born to short, primiparous, or thin mothers "normally" or "abnormally" small?* J Pediatr, 2007. 150(6): p. 603-7, 607 e1-3.
57. Leary, S., et al., *Geographical variation in relationships between parental body size and offspring phenotype at birth.* Acta Obstet Gynecol Scand, 2006. 85(9): p. 1066-79.
58. Kramer, M.S., et al., *US Black vs White disparities in foetal growth: physiological or pathological?* Int J Epidemiol, 2006. 35(5): p. 1187-95.
59. Bryan, S.M. and P.C. Hindmarsh, *Normal and abnormal fetal growth.* Horm Res, 2006. 65 Suppl 3: p. 19-27.
60. Saudino, K.J., *Behavioral genetics and child temperament.* J Dev Behav Pediatr, 2005. 26(3): p. 214-23.

3.2

Fetal circulatory redistribution and child behavior problems



Abstract

Intrauterine growth restriction has been linked to behavioral problems. While typically only birth weight as indicator of an adverse fetal environment is examined, the present study assessed fetal circulatory redistribution, also referred to as the 'brain-sparing effect', which is a fetal adaptive reaction to placental insufficiency. The aim was to investigate whether fetal circulatory redistribution protects from infant behavioral problems. Within a population-based cohort, the pulsatility indices of the umbilical artery, and of the middle and anterior cerebral arteries were measured in late pregnancy. Umbilical/cerebral ratios of pulsatility indices were related to behavioral problems, as measured by the Child Behavior Checklist, in 941 18-month-old toddlers. The umbilical/anterior cerebral ratio was associated with total problems (OR per standard deviation increase: 1.2; 95% confidence interval: 1.0, 1.5). Children with higher umbilical/anterior cerebral ratio had higher risk of internalizing problems, emotional reactivity, somatic complaints, and attention problems. A high umbilical/middle cerebral ratio was related to a higher score on the internalizing scale and on the syndrome scale somatic complaints. In conclusion, fetuses with circulatory redistribution are more likely to encounter behavioral problems. This suggests that 'brain sparing' does not completely spare the brain, but indicates an underlying pathology with consequences for behavioral outcome.

Introduction

An adverse intrauterine environment may have lifelong consequences for the developing fetus. A non-optimal environment in utero is not only related to somatic disease such as hypertension, cardiovascular disease, or non-insulin dependent diabetes mellitus in adulthood [1], but also to psychiatric disorders. Studies of the Dutch or Chinese famines showed that maternal undernutrition during pregnancy is associated with a higher risk of schizophrenia [2, 3], antisocial personality disorder [4], and depression in adulthood [5]. Moreover, several population-based studies described relations between low birth weight, a proxy for an adverse intrauterine environment, and behavior and cognition in childhood, adolescence, and adulthood [6-9]. Furthermore, animal studies and studies in clinical samples showed that intrauterine growth restriction is associated with alterations in brain tissue volumes [10, 11], and with disabilities in fine and gross motor skills, cognitive function, concentration, attention, and mood [12-14].

Intrauterine growth restriction, and consequently a lower birth weight, is most commonly caused by placental insufficiency [15]. Any disturbance in the placental-fetal circulation potentially affects the supply of nutrients such as oxygen, glucose, and amino acids [16]. Animal studies showed that in the presence of intrauterine growth restriction due to placental insufficiency, the central nervous system is preferentially perfused. This adaptation is intended to maintain oxygen supply to the brain as much as possible [17, 18]. In humans, this fetal adaptive mechanism, often referred to as the 'brain-sparing effect', can be demonstrated with Doppler ultrasound. A raised pulsatility index in the umbilical artery identifies fetuses that are growth-restricted due to placental dysfunction. It reflects a reduction in the number of arterioles in the stem villi, in combination with problems of infarction and thrombosis [19]. However, umbilical artery Doppler measurement alone is not sufficient to conclude that the blood supply is compromised [20]. The fetus adapts to placental insufficiency by vasodilatation of the cerebral circulation, which can be detected as a decreased pulsatility index in the cerebral arteries [16]. Thus, the indicator of the 'brain-sparing effect' is a raised ratio between the umbilical artery pulsatility index and the middle cerebral artery pulsatility index (the U/C ratio) [21]. The term 'brain-sparing', however, may be misleading. It refers to relative protection of the brain as compared to other organs during fetal development, but does not guarantee normal development after birth. Scherjon et al. showed in a clinical sample of preterms, many of whom were intrauterine growth restricted, that brain-sparing predicts cognitive deficits at the age of 5 years [22]. Experimentally induced placental insufficiency in animals altered brain structure in the offspring, causing reduced brain weight, ventriculomegaly, and volumetric reductions in the basal ganglia and the hippocampus [23].

In fetuses with suspected chronic hypoxia, it has been shown that Doppler signs of fetal redistribution were much more frequent in the anterior cerebral artery than in the middle cerebral artery [24], and that these signs of brain sparing in the anterior cerebral artery pre-

dicted perinatal mortality better. Dubiel et al. postulated that redistribution of fetal cerebral blood flow in the presence of chronic hypoxia aims to protect the frontal lobes, which are mainly supplied by the anterior cerebral artery, rather than the lateral and occipital parts of the brain that are supplied by the middle and posterior cerebral arteries [24]. The frontal cortex of the brain is involved in higher-order brain functions like emotional, cognitive, and motivational processes [25, 26], while the lateral and occipital lobes are mainly involved in motor and visual function. Whether fetal redistribution in the main supply route of the frontal region is sufficient to protect the fetus' cognitive and emotional development, is unclear.

We examined the hypothesis that changes in blood flow in the middle and anterior cerebral artery are associated with behavioral and emotional problems in a population-based cohort. Doppler ultrasound was used to measure U/C ratios for umbilical artery with both anterior and middle cerebral arteries. We investigated whether raised U/C ratios were related to an increased risk of behavioral problems.

Materials and methods

Setting

The study was conducted within the Generation R Focus Study, a population-based prospective cohort from fetal life until young adulthood in Rotterdam, the Netherlands [27]. Detailed prenatal ultrasound assessments were conducted in this subgroup of 1,232 Dutch pregnant women, which is ethnically homogeneous to exclude confounding or effect modification by ethnicity. All children were born between February 2003 and August 2005 and form a prenatally enrolled birth cohort. The study was conducted in accordance with the guideline proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all adult participants.

Study population

A total of 1,232 women and their children were enrolled in the Generation R Focus Study during pregnancy. We excluded twin pregnancies (n=15) and pregnancies leading to perinatal death (n=2). Moreover, we randomly excluded one child of each sibling pair (n=19) to avoid bias due to paired data. Of the remaining 1,196 fetuses, interpretable Doppler measurement of the umbilical artery and cerebral arteries was performed in 1,135 fetuses. Information on behavioral and emotional problems at age 18 months was available in 941 (83%) of these children.

Fetal circulation

Three trained sonographers carried out fetal ultrasound examinations at the visit to the research center in late pregnancy. The median (95 percent range) gestational age at ultrasound examinations was 30 (28 – 33) weeks. Online measurements used for the present study included Doppler measurements of the umbilical artery and the anterior and middle cerebral arteries [28]. Color imaging was used to optimize placement of the pulsed wave Doppler gate. For each measurement three consecutive uniform waveforms were recorded by pulsed Doppler ultrasound, during fetal apnea and without fetal movement. The mean of three measurements was used for further analysis. The umbilical artery pulsatility index was measured in a free-floating loop of the umbilical cord. The redistribution of blood flow in favor of the fetal brain was quantified by the middle and anterior cerebral artery pulsatility indices. The Doppler measurements were performed with color Doppler visualization of the circle of Willis in the fetal brain. Flow velocity waveforms were obtained in the proximal part of the cerebral arteries. For reliability analyses, intraclass correlation coefficients between and among observers were calculated in 12 subjects. Intra-observer intraclass correlation coefficients varied from 0.93 to 0.98, and inter-observer intraclass correlation coefficients varied from 0.82 to 0.91 for the Doppler measurements [28]. All ultrasound exams were performed using an ATL-Philips Model HDI 5000 (Seattle, Washington, USA) equipped with a 5.0 MHz, high frequency curved array transducer. In line with other reports on fetal brain sparing [21, 22, 29-32], we calculated the U/C ratios by dividing the pulsatility index of the umbilical artery by the pulsatility index of one of the cerebral arteries.

Child behavioral and emotional problems

The Child Behavior Checklist for toddlers was used to obtain standardized parent reports of children's problem behaviors. This questionnaire contains 99 problem items, which are scored on seven empirically based syndromes that were derived by factor analyses: Emotionally Reactive, Anxious/Depressed, Somatic Complaints, Withdrawn, Sleep problems, Attention Problems, and Aggressive Behavior. The broadband scale Internalizing is the sum score of items in the first four syndrome scales, whereas the Externalizing scale is the sum score of Attention Problems and Aggressive Behavior. The sum of all 99 problem items is the Total Problems score. Each item is scored 0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true, based on the preceding two months. Good reliability and validity have been reported for the Child Behavior Checklist [33].

Covariates

Gestational age at birth, birth weight, gender, Apgar score 1 and 5 minutes after birth, and umbilical artery cord blood pH of the infant were obtained from midwife and hospital registries at birth. We established gestational age by using the fetal ultrasound examinations within the Generation R Study. Maternal age, maternal height, and maternal educational level were determined at enrolment. To assess maternal psychopathology in mid-pregnancy, we used the Brief Symptom Inventory [34, 35]. Maternal smoking and alcohol use during pregnancy were assessed using three prenatal questionnaires at 12, 20, and 30 weeks of gestation. Postnatal questionnaires at ages 6, 12, and 24 months provided information on breast feeding, child's hospitalization and visits to medical doctors.

Statistical analyses

We calculated standard deviation scores of the U/C ratios for the middle and the anterior cerebral arteries. The Child Behavior Checklist broadband and syndrome scales were dichotomized, since the resulting scores were right-skewed and could not be transformed to satisfy the assumption of normality. Primarily, we defined a non-optimal score as the highest 15 percent of problem item scores. Additionally, we report the results for Total Problems when using the highest 25 percent and highest 10 percent as a cut-off for behavioral problems to test consistency.

Differences in baseline characteristics between children in the top 15% of Total behavioral Problems and children without behavioral problems were compared with independent t-tests for continuous normally distributed variables, Mann-Whitney U tests for continuous non-normally distributed variables, and chi-square statistics for categorical variables.

We used logistic regression models to test the associations between standard deviation scores of measures of fetal circulatory redistribution and behavioral problems.

All associations were adjusted for gender and age. The other covariates were selected as a result of exploratory analyses and included in the models if the effect estimate changed meaningfully (defined as more than 5 percent). Measures of association are presented with their 95 percent confidence intervals (CI). Statistical analyses were carried out using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

Response analysis

Analyses of missing behavioral outcome showed that mothers of children without information on behavior were 2.1 (95 percent CI: 1.3, 2.8, t=5.2, $p<0.001$) years younger, lower educated (percent university level 25.1 vs. 39.1, $\chi^2=13(1)$, $p<0.001$), more often continued smoking

during pregnancy (26.4 percent vs. 11.2 percent, χ^2=32(1), p<0.001) and had higher total psychopathology symptom scores (median 0.1, 95 percent range 0 – 1.4 vs. 0.1, 95 percent range 0 – 0.7, Mann-Whitney U, p=0.003) than mothers of children with behavioral information. Children with and without behavioral information did not differ in blood flow parameters of umbilical and cerebral arteries, birth weight, gestational age at birth and hospitalization.

Results

Table 1 compares the demographic characteristics and potential confounding variables of the 136 children who showed problem behavior at the age of 18 months and the 805 children who had no behavioral problems. Of the total sample, 51 percent was male. Children with a high score on Total Problems were more often the first-born child (71.3 percent vs. 62.5 percent, χ^2=4(1), p=0.05) compared to children with a normal score on Total Problems. Maternal psychopathology was also significantly higher in children with behavioral problems compared to children without behavioral problems.

Table 2 shows the relation between fetal circulatory redistribution and the broadband scales of the Child Behavior Checklist. The U/C ratio of the anterior cerebral artery was related

Table 1. Subject characteristics

	No behavioral problems N=805	Behavioral problems (top 15% Total Problems) N=136	*p*-value
Child			
Gestational age, weeks: median (95% range)	40.3 (36.0 – 42.4)	40.4 (37.0 – 42.6)	0.4
Birth weight, grams: mean (SD)	3512 (528)	3535 (535)	0.2
Small-for-gestational-age, % <= -1 SD	13.0	13.2	0.9
Boys, %	50.0	56.6	0.2
Age, months: mean (SD)	18.2 (0.9)	18.2 (0.6)	0.7
First-born child, %	62.5	71.3	0.05
Hospitalization during first 18 months, %	18.5	25.0	0.1
Regular GP-visits (>= 3 per 6 months), %	28.2	35.6	0.1
Outpatient clinic visits (>= 2 per 6 months), %	24.8	30.1	0.2
Mother			
Maternal age, years: mean (SD)	32.0 (3.8)	31.5 (3.7)	0.2
Maternal height, cm: mean (SD)	171.1 (6.2)	170.6 (6.9)	0.4
Maternal educational level, % university level	39.7	35.6	0.4
Maternal smoking in pregnancy, % yes	11.5	9.6	0.5
Maternal alcohol use in pregnancy, % yes	56.4	56.3	1.0
Maternal psychopathology: median (95% range)	0.10 (0.00 – 0.64)	0.17 (0.00 – 1.00)	< 0.001

Values are means (SD = standard deviations) for continuous, normally distributed variables, medians (95% range) for continuous non-normally distributed variables, and percentages for categorical variables. P-values are derived from independent t-tests for continuous normally distributed variables, Mann-Whitney U tests for continuous non-normally distributed variables, or chi-square tests for categorical variables.

Table 2. Associations between fetal circulatory redistribution and broadband scales of child behavioral problems

Measures of fetal circulatory redistribution	Total Problems		Internalizing		Externalizing	
	OR	95% CI	OR	95% CI	OR	95% CI
U/C ratio middle cerebral artery (per SD)	1.15	0.96, 1.38	1.23	1.02, 1.47*	1.10	0.92, 1.31
U/C ratio anterior cerebral artery (per SD)	1.23	1.01, 1.50*	1.25	1.03, 1.52*	1.14	0.94, 1.39

* $p < 0.05$, ** $p < 0.01$

Values are odds ratios (95% confidence intervals) from logistic regression models, adjusted for gender, age, gestational age at birth, birth weight, birth order, maternal educational level, maternal psychopathology, and child hospitalization / Medical Doctor-consultations.

OR=odds ratio, CI=confidence interval, SD=standard deviation

U/C ratio = pulsatility index umbilical artery / pulsatility index cerebral artery

to Total Problems, and to Internalizing problems. A higher U/C ratio of the middle cerebral artery was only related to a higher score on the broadband scale Internalizing. Fetal circulatory redistribution did not predict Externalizing problems. Confounding variables that changed the effect estimates more than 5 percent, were gestational age at birth, birth weight, birth order, maternal educational level, maternal psychopathology, and doctor consultations. Birth weight changed the effect estimate meaningfully, but was not an intermediate in the association between brain sparing and behavioral problems, since it was positively associated with behavioral problems (table 1). Maternal smoking, a risk factor for increased vascular resistance in the placenta and the brain [28], was not related to behavioral outcome in the fully adjusted model and did not change the effect estimates of fetal circulatory redistribution. Additionally, we examined the association of the single artery pulsatility indices with behavioral outcome, and found no associations between the pulsatility index of the umbilical artery and problem behavior. The pulsatility indices of the middle and anterior cerebral arteries were negatively associated with Internalizing (OR per SD increase in pulsatility index of both middle and anterior cerebral artery 0.8, 95 percent CI: 0.7, 1.0, $p<0.05$), but not significantly associated with Total Problems or Externalizing. When Total Problems was dichotomized at the 75th or 90th percentile, the odds ratio per standard deviation increase in U/C ratio of the anterior cerebral artery was 1.2 (95 percent CI: 1.0, 1.4, p = 0.03) and 1.2 (95 percent CI: 1.0, 1.6, p = 0.09), respectively.

Next, we analyzed the association between fetal circulatory redistribution and specific syndrome scales of the Child Behavior Checklist. Table 3 provides the odds ratios for scoring high on syndrome scales per standard deviation increase in U/C ratios. Fetal redistribution to the anterior cerebral artery was significantly related to three subscales of the Child Behavior Checklist. Per standard deviation increase in U/C ratio, the odds for having high scores on Emotionally Reactive were 26 percent higher. Secondly, a higher U/C ratio of the anterior cerebral artery also made it more likely that infants had Attention Problems or Somatic Complaints, the increase in odds was 27 percent and 23 percent, respectively. Fetal redistribution

Table 3. Associations between fetal circulatory redistribution and syndrome scales of child behavioral problems

Measures of fetal circulatory redistribution	Internalizing syndrome scales							
	Emotionally Reactive		Anxious / Depressed		Somatic complaints		Withdrawn	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
U/C ratio MCA (per SD)	1.06	0.90, 1.25	1.07	0.92, 1.25	1.23	1.03, 1.47*	1.07	0.94, 1.23
U/C ratio ACA (per SD)	1.26	1.06, 1.51**	1.07	0.91, 1.27	1.23	1.01, 1.49*	1.13	0.97, 1.31
	Externalizing syndrome scales							
	Attention problems		Aggressive behavior					
	OR	95% CI	OR	95% CI				
U/C ratio MCA (per SD)	1.14	0.97, 1.35	1.08	0.90, 1.30				
U/C ratio ACA (per SD)	1.27	1.06, 1.52**	1.11	0.91, 1.35				

* $p < 0.05$, ** $p < 0.01$

Values are odds ratios (95% confidence intervals) from logistic regression models, adjusted for gender, age, gestational age at birth, birth weight, birth order, maternal educational level, maternal psychopathology, and child hospitalization / Medical Doctor-consultations.

OR=odds ratio, CI=confidence interval, SD=standard deviation

U/C ratio = pulsatility index umbilical artery / pulsatility index cerebral artery.

MCA = middle cerebral artery, ACA = anterior cerebral artery.

to the middle cerebral artery was only related to Somatic Complaints (OR per standard deviation increase in U/C ratio: 1.23, 95 percent CI: 1.02, 1.47). Doctor-consultation only marginally accounted for this association (OR without adjustment for child hospitalization and consultation of medical doctors: 1.24, 95 percent CI: 1.04, 1.47).

Discussion

The main finding of this study was that signs of fetal brain sparing were associated to infant problem behavior. Children with fetal circulatory redistribution to the anterior cerebral artery had a higher risk of Total Problems and Internalizing problems at the age of 18 months. This preferential perfusion of the frontal lobes during pregnancy was also related to higher scores on the syndrome scales Emotional Reactivity, Somatic Complaints, and Attention Problems. The association between preferential perfusion of the middle cerebral artery and problem behavior was less evident.

Studies on fetal programming frequently used low birth weight as a proxy for an adverse intrauterine environment [7, 8, 36]. However, being born with a low birth weight or small-for-gestational-age can also be the result of constitutional factors [37]. A small fetus as a result of reduced placental perfusion and chronic hypoxia during pregnancy can be better identified using blood flow parameters of the umbilical artery and the cerebral arteries [24].

Several studies showed that Doppler signs of fetal circulatory redistribution were associated with perinatal complications such as emergent cesarean sections, low gestational age at birth, and longer stays on neonatal intensive care units [32, 38, 39]. Animal studies provided information on abnormalities in brain structure and function after unilateral ligation of the maternal uterine artery [10, 23, 40]. These studies showed that signs of the brain-sparing effect can identify fetuses at risk for perinatal morbidity and mortality, and for adverse neurodevelopmental outcome. This work suggests that the goal, to prevent cerebral hypoxia, cannot always be achieved by brain sparing. In other words, the brain is protected at the cost of other organs, but fetal circulatory redistribution does not necessarily spare the brain in absolute terms.

Earlier studies directly related blood flow parameters of brain sparing to visual, neuromotor, and cognitive outcome in clinical populations. Scherjon et al. found that children with fetal brain-sparing may have a accelerated myelination of visual pathways during the first year of life [29], but could not demonstrate a difference in visual functioning at age 11 years [31]. Moreover, although gross neuromotor outcome at age 3 years was not affected in this clinical cohort by fetal circulatory redistribution [30], brain-sparing had a detrimental effect on cognitive outcome at 5 years of age [22]. These findings seems contradictory, but may also reflect that chronic fetal hypoxia adversely affects specific brain regions, whereas other regions are spared.

The frontal cortex is involved in emotional, cognitive and motivational processes [25, 26]. Dubiel et al. suggested that the redistribution of fetal cerebral flood flow in the presence of chronic hypoxia preferentially protects the frontal region of the brain rather than the lateral and posterior parts of the brain that are responsible for motor control and sensory function. Our findings show that signs of brain sparing in the anterior cerebral artery were related to a higher risk of attention problems and emotional reactivity. Problems in attention and emotion regulation may indicate a less mature frontal-subcortical circuitry [25]. This suggests that, despite the fetal adaptive mechanism to maintain oxygen supply to brain regions that are involved in higher-order brain functions, fetal chronic hypoxia adversely affects development in this brain area.

The population-based setting of our study enabled us to assess fetal circulation physiology over the entire range of fetal weight, rather than only in fetuses with growth restriction or other obstetric complications. However, some methodological considerations need to be discussed. Selective attrition of healthier mothers could lead to bias. Although mothers of non-participating children were younger, lower educated, and more likely to report symptoms of psychopathology, we found no indication that children with intrauterine growth restriction or raised umbilical-cerebral ratios were more often lost to follow-up. However, we should be cautious in generalizing our findings to other populations. Second, using a parent-report measure to assess toddler behavior might introduce reporter bias. However, mothers were blind to their child's signs of fetal brain sparing, and we thus assume this type

of misclassification not dependent on the determinant. Furthermore, we adjusted our associations for maternal symptoms of psychopathology, which are known to color the report of actual child behavior [41]. Third, in observational studies like this, residual confounding is a potential limitation. We could rule out that our associations were explained by prematurity, sociodemographic variables, maternal smoking during pregnancy or medical complications after birth. However, there might be e.g. other environmental or common genetic factors that underlie placental perfusion or the fetal adaptive mechanism to chronic hypoxia, and predispose the child to behavioral and emotional problems.

In conclusion, the brain sparing mechanism does not protect the child from all neurodevelopmental problems that arise from an adverse fetal environment. Fetuses who experience circulatory redistribution with preferential perfusion of the brain are more likely to have behavioral and emotional problems in young childhood. Placental insufficiency can have lasting neurodevelopmental consequences. Our findings suggest that, even in children without overt intrauterine growth restriction, the umbilical-anterior cerebral ratio is a sensitive marker of chronic hypoxia and nutrient deficiency and a risk-indicator for behavioral outcome. Further research is needed to study long-term consequences of fetal brain sparing for cognitive and behavioral development at preschool and school age.

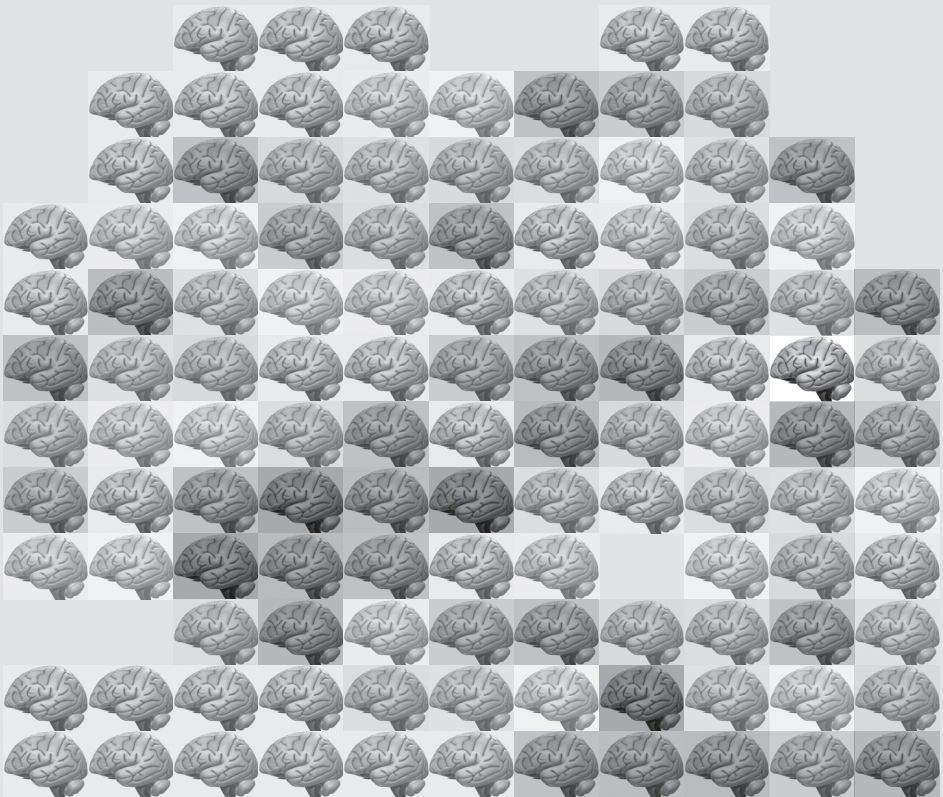
References

1. Barker, D.J., *Fetal origins of coronary heart disease.* BMJ, 1995. 311(6998): p. 171-4.
2. Susser, E., et al., *Schizophrenia after prenatal famine. Further evidence.* Arch Gen Psychiatry, 1996. 53(1): p. 25-31.
3. St Clair, D., et al., *Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959-1961.* Jama, 2005. 294(5): p. 557-62.
4. Neugebauer, R., H.W. Hoek, and E. Susser, *Prenatal exposure to wartime famine and development of antisocial personality disorder in early adulthood.* Jama, 1999. 282(5): p. 455-62.
5. Brown, A.S., et al., *Further evidence of relation between prenatal famine and major affective disorder.* Am J Psychiatry, 2000. 157(2): p. 190-5.
6. Gunnell, D., et al., *The association of fetal and childhood growth with risk of schizophrenia. Cohort study of 720,000 Swedish men and women.* Schizophr Res, 2005. 79(2-3): p. 315-22.
7. Wiles, N.J., et al., *Birth weight and psychological distress at age 45-51 years: results from the Aberdeen Children of the 1950s cohort study.* Br J Psychiatry, 2005. 187: p. 21-8.
8. Costello, E.J., et al., *Prediction from low birth weight to female adolescent depression: a test of competing hypotheses.* Arch Gen Psychiatry, 2007. 64(3): p. 338-44.
9. Nigg, J.T. and N. Breslau, *Prenatal smoking exposure, low birth weight, and disruptive behavior disorders.* J Am Acad Child Adolesc Psychiatry, 2007. 46(3): p. 362-9.
10. Mallard, C., et al., *Reduced number of neurons in the hippocampus and the cerebellum in the postnatal guinea-pig following intrauterine growth-restriction.* Neuroscience, 2000. 100(2): p. 327-33.
11. Rees, S. and T. Inder, *Fetal and neonatal origins of altered brain development.* Early Hum Dev, 2005. 81(9): p. 753-61.
12. Geva, R., et al., *Memory functions of children born with asymmetric intrauterine growth restriction.* Brain Res, 2006. 1117(1): p. 186-94.
13. Tolsa, C.B., et al., *Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction.* Pediatr Res, 2004. 56(1): p. 132-8.
14. Leitner, Y., et al., *Neurodevelopmental outcome of children with intrauterine growth retardation: a longitudinal, 10-year prospective study.* J Child Neurol, 2007. 22(5): p. 580-7.
15. Kingdom, J., et al., *Development of the placental villous tree and its consequences for fetal growth.* Eur J Obstet Gynecol Reprod Biol, 2000. 92(1): p. 35-43.
16. Baschat, A.A., *Fetal responses to placental insufficiency: an update.* Bjog, 2004. 111(10): p. 1031-41.
17. Cohn, H.E., et al., *Cardiovascular responses to hypoxemia and acidemia in fetal lambs.* Am J Obstet Gynecol, 1974. 120(6): p. 817-24.
18. Sheldon, R.E., et al., *Redistribution of cardiac output and oxygen delivery in the hypoxemic fetal lamb.* Am J Obstet Gynecol, 1979. 135(8): p. 1071-8.
19. Chauhan, S.P. and E.F. Magann, *Screening for fetal growth restriction.* Clin Obstet Gynecol, 2006. 49(2): p. 284-94.
20. Harrington, K., K. Hecher, and S. Campbell, *The fetal haemodynamic response to hypoxia*, in *A colour atlas of Doppler ultrasonography in obstetrics: an introduction to its use in maternal fetal medicine*, K. Herrington and S. Campbell, Editors. 1995, Edward Arnold: London. p. 81 - 94.
21. Scherjon, S.A., et al., *Fetal and neonatal cerebral circulation: a pulsed Doppler study.* J Perinat Med, 1992. 20(1): p. 79-82.
22. Scherjon, S., et al., *The discrepancy between maturation of visual-evoked potentials and cognitive outcome at five years in very preterm infants with and without hemodynamic signs of fetal brain-sparing.* Pediatrics, 2000. 105(2): p. 385-91.
23. Rehn, A.E., et al., *An animal model of chronic placental insufficiency: relevance to neurodevelopmental disorders including schizophrenia.* Neuroscience, 2004. 129(2): p. 381-91.
24. Dubiel, M., G.O. Gunnarsson, and S. Gudmundsson, *Blood redistribution in the fetal brain during chronic hypoxia.* Ultrasound Obstet Gynecol, 2002. 20(2): p. 117-21.

25. Alvarez, J.A. and E. Emory, *Executive function and the frontal lobes: a meta-analytic review.* Neuropsychol Rev, 2006. 16(1): p. 17-42.
26. Krain, A.L. and F.X. Castellanos, *Brain development and ADHD.* Clin Psychol Rev, 2006. 26(4): p. 433-44.
27. Jaddoe, V.W., et al., *The Generation R Study: Design and cohort profile.* Eur J Epidemiol, 2006. 21(6): p. 475-84.
28. Verburg, B.O., et al., *Fetal hemodynamic adaptive changes related to fetal growth.* Circulation, 2007. in press.
29. Scherjon, S.A., et al., *Fetal brain sparing is associated with accelerated shortening of visual evoked potential latencies during early infancy.* Am J Obstet Gynecol, 1996. 175(6): p. 1569-75.
30. Scherjon, S.A., et al., *Neurodevelopmental outcome at three years of age after fetal 'brain-sparing'.* Early Hum Dev, 1998. 52(1): p. 67-79.
31. Kok, J.H., et al., *Visual function at 11 years of age in preterm-born children with and without fetal brain sparing.* Pediatrics, 2007. 119(6): p. e1342-50.
32. Makhseed, M., et al., *Middle cerebral artery to umbilical artery resistance index ratio in the prediction of neonatal outcome.* Int J Gynaecol Obstet, 2000. 71(2): p. 119-25.
33. Achenbach, T.M. and L.A. Rescorla, *Manual for the ASEBA Preschool Forms & Profiles.* 2000, Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families.
34. de Beurs, E., *Brief Symptom Inventory, handleiding.* 2004: Leiden, the Netherlands.
35. Derogatis, L.R. and N. Melisaratos, *The Brief Symptom Inventory: an introductory report.* Psychol Med, 1983. 13(3): p. 595-605.
36. Kelly, Y.J., et al., *Birthweight and behavioural problems in children: a modifiable effect?* Int J Epidemiol, 2001. 30(1): p. 88-94.
37. Bryan, S.M. and P.C. Hindmarsh, *Normal and abnormal fetal growth.* Horm Res, 2006. 65 Suppl 3: p. 19-27.
38. Vergani, P., et al., *Antenatal predictors of neonatal outcome in fetal growth restriction with absent end-diastolic flow in the umbilical artery.* Am J Obstet Gynecol, 2005. 193(3 Pt 2): p. 1213-8.
39. Sterne, G., L.E. Shields, and T.J. Dubinsky, *Abnormal fetal cerebral and umbilical Doppler measurements in fetuses with intrauterine growth restriction predicts the severity of perinatal morbidity.* J Clin Ultrasound, 2001. 29(3): p. 146-51.
40. Tashima, L., et al., *Prenatal influence of ischemia-hypoxia-induced intrauterine growth retardation on brain development and behavioral activity in rats.* Biol Neonate, 2001. 80(1): p. 81-7.
41. Youngstrom, E., C. Izard, and B. Ackerman, *Dysphoria-related bias in maternal ratings of children.* J Consult Clin Psychol, 1999. 67(6): p. 905-16.

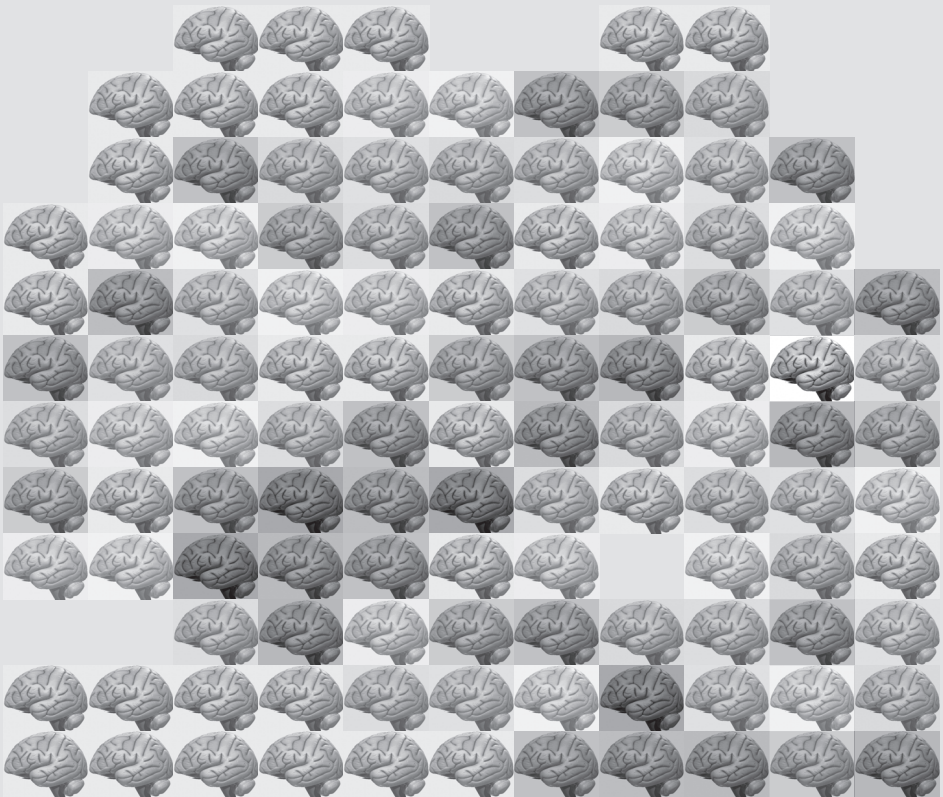
Chapter 4

Cerebral ventricular volume in infants



4.1

Fetal growth and cerebral ventricular volume



Abstract

The cerebral ventricular system is a marker of brain development and a predictor of neurodevelopmental outcome. In premature or dysmature neonates, neuroanatomical structures including the ventricular system appear to be altered. The present study aims to provide information on the association between fetal growth and neonatal cerebral ventricular size in the normal population. Within the Generation R Study, a population-based cohort study, we used three-dimensional cranial ultrasound to determine lateral ventricular volume in 778 term infants aged 4 - 12 weeks. Fetal growth characteristics were repeatedly measured in early, mid- and late pregnancy and analyzed in relation to ventricular volume divided by head circumference. Results revealed positive associations between fetal head circumference in late pregnancy and log-transformed ventricular volume (β=0.077, 95% confidence interval (0.017; 0.136), equivalent to a 7.7% increase in ventricular volume per standard deviation of head circumference). Similarly, per week longer gestational duration, ventricular volume in infancy was 6.0% larger. Multilevel modeling demonstrated that reduced growth of fetal head circumference and biparietal diameter during pregnancy were associated with decreased ventricular volume in infancy. In conclusion, fetal maturation is positively associated to cerebral ventricular size in term infants. Larger ventricular size in term infants needs to be distinguished from ventricular enlargement due to intraventricular hemorrhage or white matter damage in premature or dysmature infants. Moreover, the naturally occurring enlargement of ventricles during infancy should be considered in interpreting reports on increased ventricular volumes in several neuropsychiatric disorders.

Introduction

The size of the cerebral ventricular system is a marker of brain development [1] and can be reliably explored by ultrasound in prenatal and early postnatal life [2, 3]. Ventricular size in infancy has mainly been studied in premature born infants [4-6]. These children with (very) short gestational duration, even those without gross evidence of brain injury, have subtle structural signs of altered brain development such as lower cortical gray matter volumes, and overall reduction in cerebral tissue volume [7-10]. However, it is unclear whether brain structure is determined by gestational duration across the entire range in the general population.

Irrespective of the degree of prematurity, small for gestational age infants are at greater risk for neurodevelopmental impairments than appropriate for gestational age infants [11]. In contrast to gestational duration, birth weight has been described to be a continuous risk factor for developmental disabilities in the general population [12]. Alterations in brain development have been described in growth-retarded children [13-15]. However, whether neuroanatomical structure is influenced by fetal growth in the normal population has not been studied.

Little is known about normal human anatomical brain development in young infancy. This lack of normative data should be addressed to interpret findings of ventricular enlargement in premature and dysmature neonates. Moreover, with the increasing amount of imaging studies addressing subtle deviations in brain development in children with neuropsychiatric disorders, including larger ventricular volumes in schizophrenia and autism spectrum disorders [16], information on normal brain development from fetal life onwards is needed to understand the pathophysiological pathways of these disorders.

Our aim was to test the hypothesis that fetal maturation, as measured by fetal growth and gestational duration, affects brain structure within a population-based sample. The present study assessed cerebral ventricular volume, using three-dimensional cranial ultrasound via the anterior fontanel in a sample of 778 children aged 4 to 12 weeks after birth.

Materials and methods

Setting

This study was conducted within a subgroup of the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood in Rotterdam, the Netherlands, which has been described previously in detail [17, 18].

In 1,232 Dutch pregnant women and their children, detailed assessments were conducted. This subgroup is ethnically homogeneous to exclude confounding or effect modification by

ethnicity. All children were born between February 2003 and August 2005 and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. The study was conducted in accordance with the guideline proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

Study population

The total of 1,232 mothers delivered 1,244 live births (15 twin pregnancies, 1 intrauterine fetal death, 2 neonatal deaths). Of these, 904 (73%) participated in the postnatal assessment at the age of 4 –12 weeks. Interpretable three-dimensional cranial ultrasounds were performed in 778 infants who visited the research center at age 4 - 12 weeks. Missing cranial ultrasounds were mainly due to movement or restlessness of the infant, a too small anterior fontanel owing to age beyond 3 months or unavailability of a trained sonographer. Postnatal head circumference was not measured in 33 children. We excluded twins (n=19) from the multilevel analyses, since growth potentials for individual fetuses in multiple pregnancies are not comparable to singleton pregnancies. Thus, analyses with gestational duration and birth weight were based on 745 subjects, whereas 726 children were included in one or more multilevel analyses.

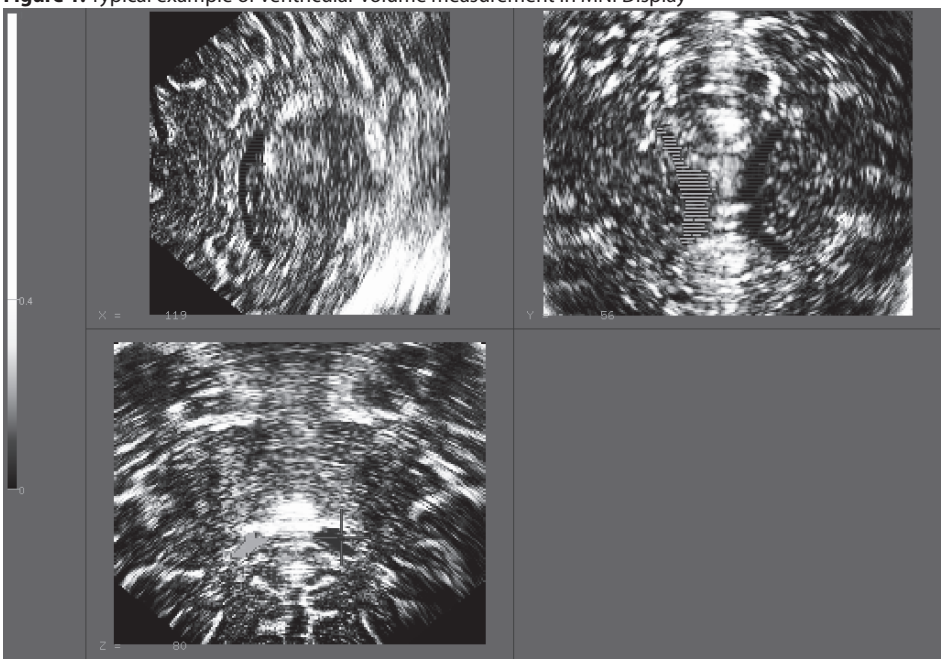
Three-dimensional imaging

Cranial ultrasound was performed with a commercially available multifrequency electronic transducer (3.7-9.3 MHz) with a scan angle of 146°, usable for two-dimensional imaging as well as for three-dimensional volume acquisition (Voluson 730 Expert, GE Healthcare, Waukesha WI, USA). Infants were situated in supine position. The probe was positioned on the anterior fontanel and a volume box was placed at the level of the foramen of Monro in a symmetrical coronal section. The volume scan was then initialized in a period without movement of the child, the transducer automatically swept for 5 seconds. A pyramid-shaped volume of brain tissue was scanned from which coronal, sagittal and axial sections were calculated.

Postprocessing

The data were transformed from v00 (three-dimensional ultrasound file format in Voluson 730) to mnc (three-dimensional file format from MNI in Montreal) and analyzed with MNI Display software (Montreal Neurological Institute, McGill University, Quebec, Canada). Four raters manually traced left and right lateral ventricles using a mouse-driven cursor, after intensive training and assessment standardization with an experienced ultrasonographer (PG). Raters were blind to subject information. The anterior boundary of the lateral ventricles

Figure 1. Typical example of ventricular volume measurement in MNI Display



was defined as the coronal plane that included the most anterior part of the lateral ventricle, additionally confirmed in axial and sagittal orientations. Tracing proceeded from anterior to posterior, at every second coronal slide. For most infants, the slices comprising the occipital and temporal horns of the lateral ventricular systems were not interpretable. To enhance validity, we therefore decided to calculate only the ventricular volume of the frontal horns, ventricular body and trigone that were visible in all images. The coronal plane where the echogenic choroid plexus protrudes into the posterior horn was used as a landmark and the posterior boundary was defined as the tenth slide prior to this point. Applying thresholds of darkness for the ventricles further standardized tracing. We quantified the number of voxels of the left and right ventricle that were traced, which was transformed to a volume using the GE-software 3D View and the original images. Figure 1 shows a typical example of ventricular volume measurement in MNI Display. For reliability analyses, the four raters each segmented 20 images twice. For the right ventricle, intraobserver intraclass correlation coefficients varied between 0.989 (95% Confidence Interval (CI): 0.958;0.997) and 0.993 (95% CI: 0.976;0.998). For the left ventricle, intraobserver intraclass correlation coefficients varied from 0.992 (95% CI: 0.979;0.997) to 0.997 (95% CI: 0.989;0.999). Twenty children were rated by all four tracers. Interobserver intraclass correlation coefficients were 0.950 (95% CI: 0.874;0.981) for the right ventricle and 0.981 (95% CI: 0.917;0.994) for the left ventricle.

Determinants

Midwife and hospital registries provided information on date of birth and birth weight. Gestational age was established using the fetal ultrasound examinations within the Generation R Study. Fetal growth measurements, i.e. head circumference, biparietal diameter, abdominal circumference and femur length, were carried out in early, mid- and late pregnancy, using standardized ultrasound procedures. Median gestational age (95% range) in early pregnancy was 13 (11 – 17) weeks, in mid-pregnancy 21 (19 – 23) weeks, and in late pregnancy 30 (28 – 33) weeks. We constructed gestational age adjusted standard deviation scores for all fetal growth measurements. The intra- and interobserver reliability of fetal biometry measurements in early pregnancy within the Generation R Study was good. Intraobserver intraclass correlation coefficients varied from 0.982 (femur length) to 0.995 (head circumference and biparietal diameter) and interobserver intraclass correlation coefficients varied from 0.980 (abdominal circumference) to 0.994 (biparietal diameter) with coefficients of variation between 1.8 and 5.9%. Atrial width of lateral ventricles, the widest diameter of the atrium of one of the lateral ventricles in an axial plane, was measured in mid- and in late pregnancy. During the same visit as the cranial ultrasound, we measured the fronto-occipital head circumference (cm) at its maximum diameter through the glabella and occiput to the nearest 0.1 cm, using a flexible measuring tape.

Covariates

Maternal age and maternal anthropometrics were assessed at enrolment in one of the research centers. The first questionnaire at enrolment provided information about maternal educational level. Maternal smoking was assessed by three questionnaires during pregnancy. Mode of delivery, gender, Apgar scores and umbilical artery cord blood pH were obtained from midwife and hospital registries at birth. We classified mode of delivery as 'caesarean section', 'spontaneous vaginal delivery' and 'instrumented vaginal delivery'. Weight of the child (grams) was measured without clothes using an electronic scale and length (cm) was measured in supine position using a neonatometer.

Statistical analyses

To examine whether non-response was selective, we compared core data of children with cranial ultrasound data to children who participated prenatally in the Generation R Focus Study but did not have valid cranial ultrasounds.

We log-transformed total ventricular volume / head circumference to obtain a normally distributed outcome variable. For the sake of simplicity, we will term our main outcome variable "ventricular volume", by which we mean "lateral ventricular volume divided by head

Table 1. Subject characteristics

	Boys (n = 406)	Girls (n=372)
Pregnancy and birth characteristics		
Maternal age, years	31.6 (4.1)	32.1 (3.8)
Maternal educational level, % high	65.3	66.7
Mid-pregnancy growth characteristics (median gestational age (95% range): 20.5 (18.7 – 22.7) weeks)		
Head circumference, mm	180.0 (13.0)	176.4 (11.6)
Biparietal diameter, mm	50.8 (3.7)	49.6 (3.5)
Abdominal circumference, mm	157.5 (14.1)	155.7 (12.1)
Femur length, mm	33.1 (3.2)	33.0 (3.0)
Atrial width of lateral ventricle, mm	5.9 (1.1)	5.6 (1.2)
Late pregnancy growth characteristics (median gestational age (95% range): 30.4 (28.4 – 32.6) weeks)		
Head circumference, mm	288.3 (11.8)	283.3 (11.3)
Biparietal diameter, mm	81.6 (4.0)	79.9 (3.9)
Abdominal circumference, mm	266.8 (16.5)	264.6 (16.2)
Femur length, mm	57.2 (3.0)	57.3 (2.9)
Atrial width of lateral ventricle, mm	5.4 (1.8)	5.1 (1.7)
Gestational age at birth, weeks	40.1 (35.7 – 42.4)	40.1 (35.6 – 42.4)
Birth weight, grams	3522 (539)	3424 (511)
Postnatal characteristics		
Height, cm	57.4 (2.5)	56.1 (2.5)
Weight, grams	5045 (706)	4696 (620)
Head circumference, mm	39.0 (1.3)	38.2 (1.4)
Volume of left lateral ventricle, ml	0.45 (0.07 – 2.11)	0.40 (0.06 – 1.54)
Volume of right lateral ventricle, ml	0.37 (0.06 – 1.35)	0.34 (0.06 – 1.15)
Total lateral ventricular volume, ml	0.84 (0.15 – 3.24)	0.76 (0.14 – 2.65)
Lateral ventricular volume / head circumference, ml/cm	0.02 (0.00 – 0.08)	0.02 (0.00 – 0.07)

Values are means (SD) for continuous normally distributed variables, medians (95% range) for continuous non-normally distributed variables, and percentages for categorical variables.

circumference", with the exception of table 1, where we pointed out differences in ventricular volume between boys and girls unadjusted and adjusted for head circumference. Results were very similar, whether lateral ventricular volume was adjusted for head circumference or not. Coefficients from multivariable linear regression for the log-transformed ventricular volumes are presented and interpreted in units of symmetric percentage differences [19]. In addition, we used longitudinal multilevel analysis [20] to analyze the associations between repeatedly measured fetal growth characteristics and infant ventricular volume at age 6 weeks. Multilevel models account for the dependency between measurements in early, mid- and late pregnancy in the same subject. The curves were fitted with random effects for both intercept and gestational age. To visualize our findings, we show the difference in standard deviation score of fetal head circumference from mid- to late pregnancy in tertiles of infant ventricular volume. All associations were controlled for core variables (gender, age, length and weight of child, maternal age), socio-economic status related confounders (maternal educational level, maternal smoking in pregnancy, maternal height), and obstetric variables (gestational duration, mode of delivery, Apgar scores 1 minute after birth, cord blood pH).

The models include both postnatal age and postnatal age^2, since the association between postnatal age and ventricular volume showed a curvilinear function. Measures of association are presented with their 95% confidence intervals (CI). Statistical analyses were carried out using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA) and SAS v.8.2 (Stata Corporation, College Station, TX, USA), including the Proc Mixed module for longitudinal multilevel analysis.

Results

General characteristics of the participating children are presented in table 1. Of this sample, 52% was male. Fetal and postnatal head circumference, birth weight, and postnatal weight and length were larger in boys than in girls. The median (95% range) age of infants was 6 (4 – 12) weeks. Analyses of missing cranial ultrasounds showed that mothers of children without valid data on lateral ventricular volumes were 0.9 (95% confidence interval (CI): 0.4; 1.4) years younger and were significantly lower educated (% higher education 56.9% vs. 65.9%, $\chi^2 = 9.3$, df = 2, p = 0.01) than mothers of children with valid cranial ultrasound data. Children who had cranial ultrasound did not differ from children in the Generation R Focus Study without cranial ultrasound information.

The lateral ventricular volume, not corrected for head circumference, was extremely variable, as can be seen in the 95% range (table 1). Boys had larger right ventricles (t=2.19, p-value = 0.03), larger left ventricles (t=2.01, p-value=0.05) and larger total ventricular volume (t=2.48, p-value = 0.01). However, total ventricular volume divided by head circumference did not significantly differ between boys and girls.

Table 2 presents the associations between gestational duration, birth weight, and mid- and late pregnancy growth characteristics and ventricular volume. Ventricular volume was 6.0% larger per week longer gestation. None of the fetal growth characteristics in mid-pregnancy, nor birth weight was associated to ventricular volume. However, larger fetal head circumference, larger biparietal diameter and larger atrial width of lateral ventricle in late pregnancy predicted larger ventricles in infancy. Per standard deviation of fetal head circumference for instance, ventricular volume was 7.7% larger. Postnatal age, one of the confounding variables, was positively and curvilinear related to ventricular volume. Figure 2 shows the scatter plot of raw data and the estimated back-transformed regression line, which represents the association between ventricular volume (not corrected for head circumference) and postnatal age after adjusting for all covariates.

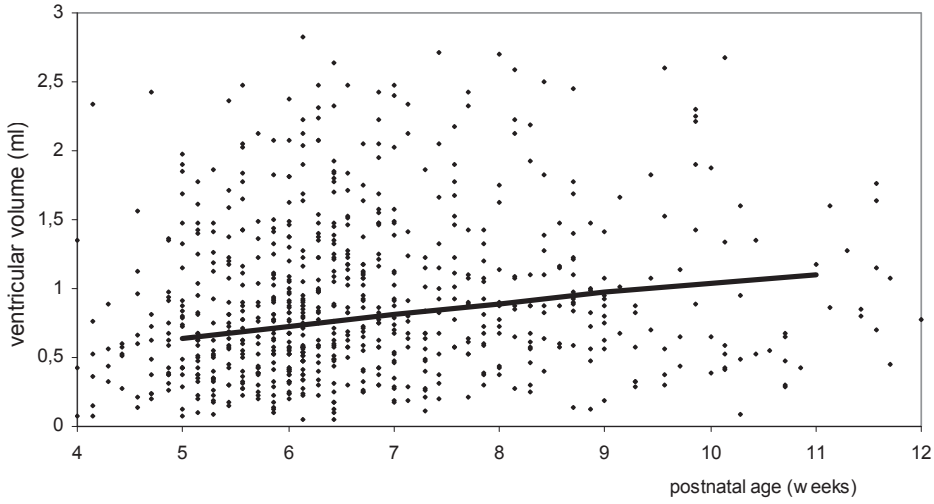
Figure 3 depicts the association between categories of gestational age at birth and mean infant ventricular volume at age 6 weeks. In our study of predominantly term infants, we found larger ventricular volumes with longer gestation.

Table 2. Determinants of infant ventricular volume

	Ventricular volume / Head circumference (log-transformed)
Gestational duration (weeks)	0.060 (0.022;0.099)**
Birth weight (kg)	0.040 (-0.121;0.201)
Mid-pregnancy growth characteristics	
Head circumference (SD)	0.047 (-0.008;0.102)
Biparietal diameter (SD)	0.040 (-0.019;0.098)
Abdominal circumference (SD)	0.001 (-0.056;0.059)
Femur length (SD)	0.021 (-0.040;0.081)
Atrial width of lateral ventricle (mm)	0.033 (-0.027;0.093)
Late pregnancy growth characteristics	
Head circumference (SD)	0.077 (0.017;0.136)*
Biparietal diameter (SD)	0.060 (0.000;0.111)*
Abdominal circumference (SD)	0.047 (-0.011;0.105)
Femur length (SD)	0.042 (-0.019;0.103)
Atrial width of lateral ventricle (mm)	0.101 (0.071;0.131)**

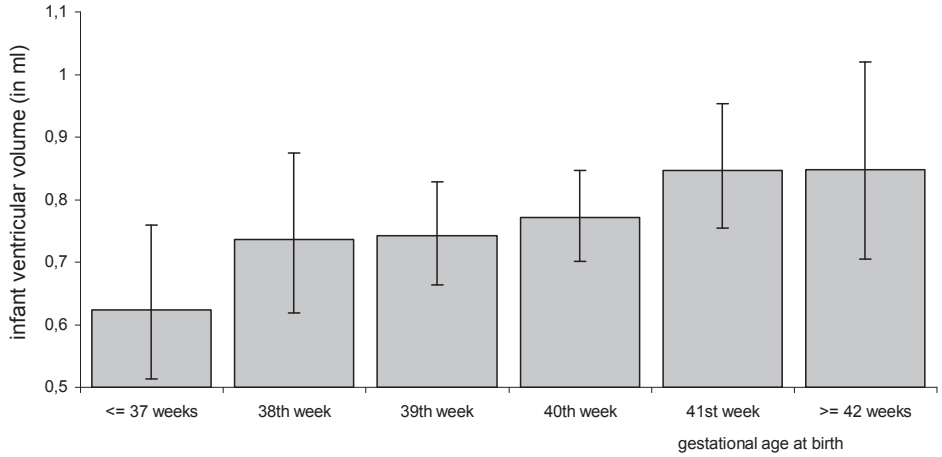
Values are regression coefficients (95% confidence interval) from linear regression and reflect the difference in log-transformed ventricular volume / head circumference per week increase of gestational duration, per kilogram increase in birth weight, per standard deviation increase in growth characteristic or per millimeter increase in atrial width of lateral ventricle. All models were adjusted for gender, age (linear and squared), maternal age, maternal educational level, maternal height, maternal smoking in pregnancy, mode of delivery, Apgar score, umbilical artery cord blood pH, height and weight of the child. The models with growth characteristics as determinants were additionally adjusted for gestational duration. *p-value < 0.05, **p-value < 0.01

Figure 2. Postnatal age and ventricular volume



Scatter plot shows relationship between ventricular volume (not corrected for head circumference) and postnatal age. The estimated back-transformed regression line, Log(ventricular volume) = -1.331 + 0.213*age – 0.0076*age*age, adjusted for gender, gestational duration, maternal age, maternal height, maternal educational level, maternal smoking in pregnancy, mode of delivery, Apgar score 1 minute after birth, umbilical artery cord blood pH, height and weight of the child, is marked in the scatter plot.

Figure 3. Gestational age at birth and ventricular volume at age 6 weeks

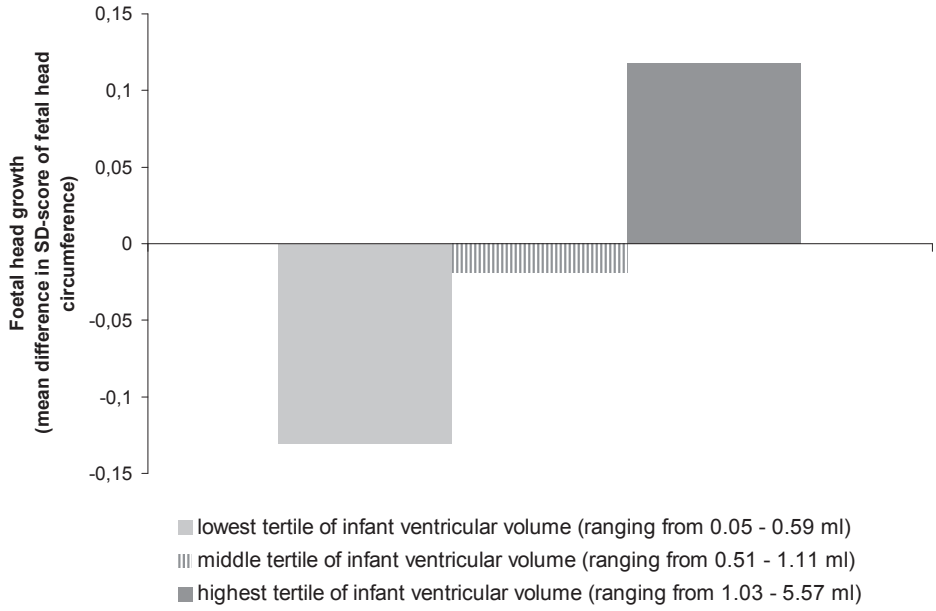


Values are estimated means of ventricular volume and upper limit 95% confidence intervals adjusted for age, gender, maternal age, maternal height, maternal educational level, maternal smoking in pregnancy, mode of delivery, Apgar score 1 minute after birth, umbilical cord blood pH, height, and weight of the child at age 4-12 weeks. Number of subjects in the categories of gestational age at birth was 67 for 37 or less weeks, 69 for birth in the 38th week of gestation, 163 for the 39th weeks, 228 for the 40th week, 153 for the 41st week, and 60 for birth after 42 weeks of gestation. In the model with continuous gestational duration, the p-value was 0.01.

Figure 4 presents the association between growth of fetal head circumference and tertiles of ventricular volume at age 6 weeks. With linear mixed models, we derived growth trajectories of fetal head circumference. Figure 4 depicts the difference in standard deviation score from mid- to late pregnancy. A negative difference in standard deviation score of fetal head circumference indicates a decreased growth velocity of the fetal head from mid- to late pregnancy, whereas a positive difference in standard deviation score indicates an increased growth velocity of the fetal head. In children with smaller ventricular volume at the age of 6 weeks, growth of fetal head circumference was significantly slower (i.e. a decrease in standard deviation score) than in infants with large ventricular volume controlled for head circumference in infancy. Results were very similar when lateral ventricular volume was not controlled for head circumference in infancy.

Similarly, increased growth of fetal biparietal diameter positively affected ventricular volume in infancy (data not shown). Growth trajectories of abdominal circumference or femur length did not influence ventricular volume. The atrial width of lateral ventricle, which represents the prenatal measure of ventricular size, decreased from mid- to late pregnancy (table 1). The largest mean decrease (from 5.4 mm in mid-pregnancy to 4.4 mm in late pregnancy) was seen in infants in the lowest tertile of postnatal ventricular volume, which was significantly larger than the mean decrease in prenatal ventricular size (5.5 to 5.4 mm from mid- to late pregnancy) of infants in the highest tertile of postnatal ventricular volume.

Figure 4. Growth of fetal head circumference from mid- to late pregnancy and ventricular volume in infants aged 6 weeks



Values are estimates based on linear mixed models and reflect the difference in SD-score of head circumference during pregnancy, adjusted for gender, postnatal age, maternal age, maternal height, maternal educational level, maternal smoking in pregnancy, mode of delivery, Apgar score 1 minute after birth, umbilical artery cord blood pH, gestational age at birth, height and weight of the child. Tertiles of ventricular volume overlap due to correction of postnatal ventricular volume for postnatal head circumference.

SD = standard deviation. A negative difference in SD-score indicates a lower growth velocity of the fetal head from mid- to late pregnancy, whereas a positive difference in SD-score indicates a higher growth velocity of the fetal head.

** $p < 0.01$

Discussion

This population-based study showed that fetal size and growth patterns as well as gestational duration determined brain structure in infancy. Larger head size and increased head growth during pregnancy predicted larger ventricular volume. Furthermore, postnatal ventricular volume was associated to the growth trajectory of prenatal ventricular size. Finally, the volume of the ventricular system was larger in children with longer gestation.

Larger ventricular volumes due to longer gestation need to be distinguished from ventricular enlargement due to premature birth or medical complications. The ventricular dilatation in preterms has been suggested to be the result of filling of the void left by the loss (or failure to growth) of tissue [21]. Especially the white matter might be damaged diffusely by premature birth. Furthermore, intraventricular hemorrhage often occurs in infants born preterm due to perinatal hypoxia-ischemia or positive pressure ventilation and causes ventricular enlargement by obstruction or absorption problems of cerebrospinal fluid. These

causes of ventricular dilatation in clinical samples of premature born infants might counteract the effects of maturity on brain development. In contrast to studies in preterms [4, 5, 22], our results in a predominantly term population show that gestational duration has a positive effect on ventricular size. Studies in children and adolescents reported that lateral ventricular volume increases across the age span 4-18 years [4, 6, 23, 24]. We show that the age-related increase in ventricular volume is marked during infancy as well. Our study thus suggests that larger ventricular size in young infants without evidence of brain injury is a sign of a more mature brain.

Like prematurity, intrauterine growth restriction leads to neurodevelopmental impairments. Several clinical studies, which have compared intrauterine growth restricted infants to healthy term infants, reported reduced volumes of total brain tissue and cortical gray matter [13-15]. Furthermore, being small for gestational age is a risk factor for major and minor neurological and psychiatric impairments [25-31].

To our knowledge, this is the first study to report a continuous association of fetal growth with structural brain morphology that holds across the entire range. Since our data cannot provide insight why fetal growth is linked to structural brain differences, we can only speculate about the underlying mechanisms. Possibly, placental insufficiency leads to an altered endocrine status with reduced levels of growth- and neurodevelopmental hormones, which could directly or indirectly influence brain development [32, 33]. Furthermore, genetic factors could explain the found associations between fetal head growth and subsequent brain structure in infancy. A substantial amount of neuronal genes is known to affect brain development in utero, both directly and in interaction with environmental factors like hypoxia during fetal development [34]. Our primary aim was not to provide reference values for ventricular volumes, since our quantification method uses study-specific landmarks. More importantly, our results give insight in the normal development of the cerebral ventricular system. The volumetric changes of the ventricular system in utero have been described by ultrasound and magnetic resonance-studies, but due to complex maturational changes in shape, increase and decrease in size with gestational age have been reported [2, 35-41]. Here, we showed a small but significant absolute decrease of mean atrial diameter throughout gestation, which we reported earlier in a sample of more than 3,000 fetuses [42]. Our two findings, a decrease of mean atrial diameter of lateral ventricle from 20 to 32 weeks of gestation and an increase of the postnatal ventricular volume with a longer gestational duration, suggests a turning point in the developing curve of the ventricular system. However, the two-dimensional measurements of the atrium and the three-dimensional measurement of the frontal horns and ventricular body after birth are not equivalent. Moreover, we were not able to map the development of the ventricular system from 32 weeks of gestation to 6 weeks postnatal. From our results, one can only conclude that a smaller size and a decreasing growth pattern of the lateral ventricle atrial width in pregnancy are predictive of smaller ventricular volume in infancy.

Our findings shed light on determinants of normal brain development during early postnatal life. The high variability in cerebral ventricular volume could be partly explained by fetal growth, gestational duration, and postnatal age. It is of note that the increase of ventricle to brain ratio is an integral part of normal brain development. On the one hand, an increased ventricle to brain ratio is frequently related to neuropsychiatric disorders, like schizophrenia, autism spectrum disorders, and eating disorders [16, 43], and interpreted as a general measure of cerebral damage. On the other hand, diminished age-related increases in lateral ventricular volumes have been described in attention-deficit hyperactivity disorder patients [44]. Further research should consider the influence of prenatal and early postnatal determinants on brain maturation when studying variations in general brain parameters in neuropsychiatric disorders.

Some methodological issues need to be discussed. Selection effects may have influenced our findings. Selective dropout of children from younger and lower educated mothers occurred. Furthermore, children in whom cranial ultrasounds were unsuccessful were on average older, which was expected, as the anterior fontanel narrows with increasing age. Maternal age and maternal educational level were not related to ventricular size in our sample, but we cannot rule out that this selective dropout impeded the detection of modest associations.

Ultrasound cannot provide information on white matter injury, nor on cerebrospinal fluid volumes beyond the ventricular system. We are therefore unable to distinguish ventricular enlargement from white matter tissue loss. However, three-dimensional ultrasound has been shown to provide a valid measure of lateral ventricular volume in infants, very similar to that obtained with magnetic resonance imaging [3]. Motion artifacts and shadows of bone tissue due to narrow anterior fontanels could have influenced the tracing of ventricular volume. However, in most children in the age 4 – 12 weeks, the anterior fontanel is quite wide and images with massive bone shadows or serious distortion were excluded. Finally, there may be operator error in the tissue segmentation. Our intra- and interobserver reliability measures were very acceptable with values of intraclass correlation coefficients > 0.95, but these high values partly reflect the high variance in ventricular volumes within our population. However, our segmentation technique is very well suited to differentiate between individuals within our population.

In conclusion, fetal maturation partly explains the wide variability of ventricular size in healthy term infants. Up till now, most studies have focused exclusively on clinical samples of premature or dysmature born infants. A better understanding of normal infant brain maturation is needed to interpret findings in premature or low birth weight children and to elucidate the pathogenesis of neuropsychiatric disorders. The consequences of smaller or larger ventricular size in term children are yet unknown. Whether ventricular volume predicts deviations in motor, cognitive, or behavioural development needs to be studied.

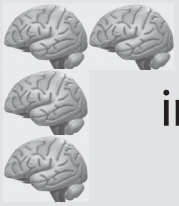
References

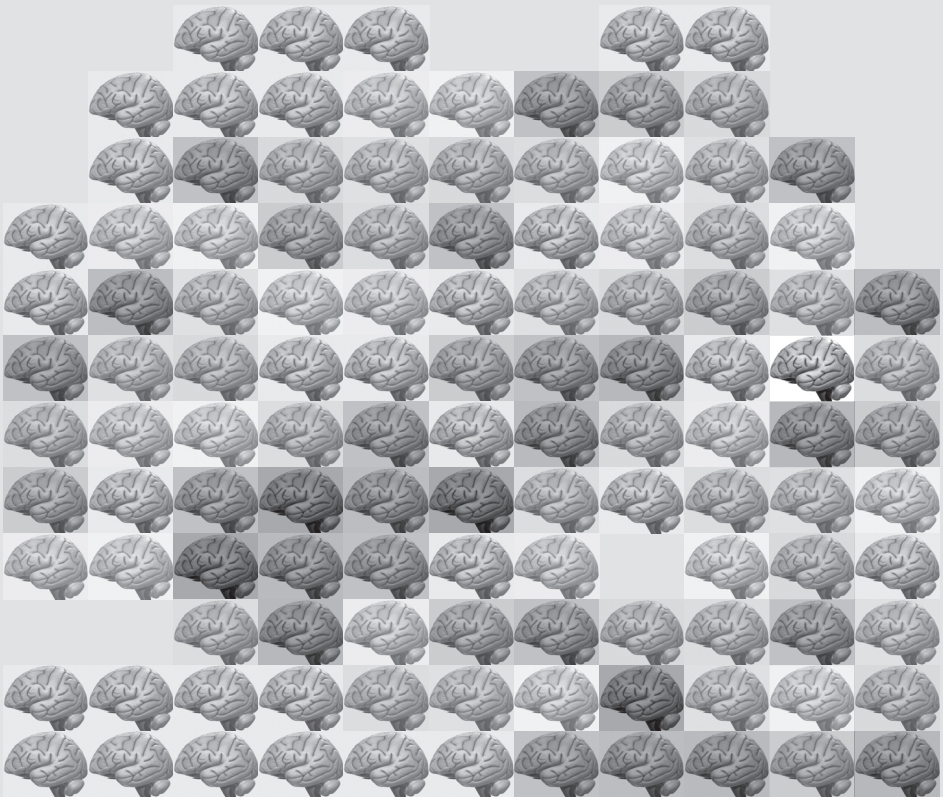
1. Gilmore, J.H., et al., *Mild ventriculomegaly detected in utero with ultrasound: clinical associations and implications for schizophrenia.* Schizophr Res, 1998. 33(3): p. 133-40.
2. Garel, C., *Fetal cerebral biometry: normal parenchymal findings and ventricular size.* Eur Radiol, 2005. 15(4): p. 809-13.
3. Gilmore, J.H., et al., *Infant cerebral ventricle volume: a comparison of 3-D ultrasound and magnetic resonance imaging.* Ultrasound Med Biol, 2001. 27(8): p. 1143-6.
4. Saliba, E., et al., *Area of lateral ventricles measured on cranial ultrasonography in preterm infants: reference range.* Arch Dis Child, 1990. 65(10 Spec No): p. 1029-32.
5. Davies, M.W., et al., *Reference ranges for the linear dimensions of the intracranial ventricles in preterm neonates.* Arch Dis Child Fetal Neonatal Ed, 2000. 82(3): p. F218-23.
6. Haiden, N., et al., *3-D ultrasonographic imaging of the cerebral ventricular system in very low birth weight infants.* Ultrasound Med Biol, 2005. 31(1): p. 7-14.
7. Peterson, B.S., et al., *A functional magnetic resonance imaging study of language processing and its cognitive correlates in prematurely born children.* Pediatrics, 2002. 110(6): p. 1153-62.
8. Kesler, S.R., et al., *Volumetric analysis of regional cerebral development in preterm children.* Pediatr Neurol, 2004. 31(5): p. 318-25.
9. Inder, T.E., et al., *Abnormal cerebral structure is present at term in premature infants.* Pediatrics, 2005. 115(2): p. 286-94.
10. MacKendrick, W., *Understanding neurodevelopment in premature infants: applied chaos theory.* J Pediatr, 2006. 148(4): p. 427-9.
11. McCarton, C.M., et al., *Cognitive and neurologic development of the premature, small for gestational age infant through age 6: comparison by birth weight and gestational age.* Pediatrics, 1996. 98(6 Pt 1): p. 1167-78.
12. van Os, J., et al., *A prospective twin study of birth weight discordance and child problem behavior.* Biol Psychiatry, 2001. 50(8): p. 593-9.
13. Huppi, P.S., et al., *Quantitative magnetic resonance imaging of brain development in premature and mature newborns.* Ann Neurol, 1998. 43(2): p. 224-35.
14. Tolsa, C.B., et al., *Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction.* Pediatr Res, 2004. 56(1): p. 132-8.
15. Mewes, A.U., et al., *Regional brain development in serial magnetic resonance imaging of low-risk preterm infants.* Pediatrics, 2006. 118(1): p. 23-33.
16. Hendren, R.L., I. De Backer, and G.J. Pandina, *Review of neuroimaging studies of child and adolescent psychiatric disorders from the past 10 years.* J Am Acad Child Adolesc Psychiatry, 2000. 39(7): p. 815-28.
17. Hofman, A., et al., *Growth, development and health from early fetal life until young adulthood: the Generation R Study.* Paediatr Perinat Epidemiol, 2004. 18(1): p. 61-72.
18. Jaddoe, V.W., et al., *The Generation R Study: Design and cohort profile.* Eur J Epidemiol, 2006. 21(6): p. 475-84.
19. Cole, T.J., *Sympercents: symmetric percentage differences on the 100 log(e) scale simplify the presentation of log transformed data.* Stat Med, 2000. 19(22): p. 3109-25.
20. Twisk, J.W.R., *Applied Multilevel Analysis. A practical Guide*. 2006, Cambridge, UK: University Press.
21. Leviton, A. and F. Gilles, *Ventriculomegaly, delayed myelination, white matter hypoplasia, and "periventricular" leukomalacia: how are they related?* Pediatr Neurol, 1996. 15(2): p. 127-36.
22. Levene, M.I., *Measurement of the growth of the lateral ventricles in preterm infants with real-time ultrasound.* Arch Dis Child, 1981. 56(12): p. 900-4.
23. Lenroot, R.K. and J.N. Giedd, *Brain development in children and adolescents: insights from anatomical magnetic resonance imaging.* Neurosci Biobehav Rev, 2006. 30(6): p. 718-29.

24. Giedd, J.N., et al., *Quantitative magnetic resonance imaging of human brain development: ages 4-18.* Cereb Cortex, 1996. 6(4): p. 551-60.
25. Berle, J.O., et al., *Outcomes in adulthood for children with foetal growth retardation. A linkage study from the Nord-Trondelag Health Study (HUNT) and the Medical Birth Registry of Norway.* Acta Psychiatr Scand, 2006. 113(6): p. 501-9.
26. Walther, F.J., *Growth and development of term disproportionate small-for-gestational age infants at the age of 7 years.* Early Hum Dev, 1988. 18(1): p. 1-11.
27. Strauss, R.S., *Adult functional outcome of those born small for gestational age: twenty-six-year follow-up of the 1970 British Birth Cohort.* JAMA, 2000. 283(5): p. 625-32.
28. Larroque, B., et al., *School difficulties in 20-year-olds who were born small for gestational age at term in a regional cohort study.* Pediatrics, 2001. 108(1): p. 111-5.
29. O'Keeffe, M.J., et al., *Learning, cognitive, and attentional problems in adolescents born small for gestational age.* Pediatrics, 2003. 112(2): p. 301-7.
30. Geva, R., et al., *Memory functions of children born with asymmetric intrauterine growth restriction.* Brain Res, 2006. 1117(1): p. 186-194.
31. Wu, Y.W., et al., *Cerebral palsy in a term population: risk factors and neuroimaging findings.* Pediatrics, 2006. 118(2): p. 690-7.
32. Mallard, E.C., et al., *Ventriculomegaly and reduced hippocampal volume following intrauterine growth-restriction: implications for the aetiology of schizophrenia.* Schizophr Res, 1999. 40(1): p. 11-21.
33. Dieni, S. and S. Rees, *Dendritic morphology is altered in hippocampal neurons following prenatal compromise.* J Neurobiol, 2003. 55(1): p. 41-52.
34. Schmidt-Kastner, R., et al., *Gene regulation by hypoxia and the neurodevelopmental origin of schizophrenia.* Schizophr Res, 2006. 84(2-3): p. 253-71.
35. Encha-Razavi, F. and P. Sonigo, *Features of the developing brain.* Childs Nerv Syst, 2003. 19(7-8): p. 426-8.
36. Rados, M., M. Judas, and I. Kostovic, *In vitro MRI of brain development.* Eur J Radiol, 2006. 57(2): p. 187-98.
37. Glonek, M., A. Kedzia, and W. Derkowski, *Prenatal assessment of ventriculomegaly: an anatomical study.* Med Sci Monit, 2003. 9(7): p. MT69-77.
38. Grossman, R., et al., *Quantitative MRI measurements of human fetal brain development in utero.* Neuroimage, 2006. 33(2): p. 463-70.
39. Kinoshita, Y., et al., *Volumetric analysis of the germinal matrix and lateral ventricles performed using MR images of postmortem fetuses.* AJNR Am J Neuroradiol, 2001. 22(2): p. 382-8.
40. Levine, D., et al., *MR imaging appearance of fetal cerebral ventricular morphology.* Radiology, 2002. 223(3): p. 652-60.
41. Prayer, D., et al., *MRI of normal fetal brain development.* Eur J Radiol, 2006. 57(2): p. 199-216.
42. Roza, S.J., et al., *Effects of maternal smoking in pregnancy on prenatal brain development. The Generation R Study.* Eur J Neurosci, 2007. 25(3): p. 611-617.
43. Eliez, S. and A.L. Reiss, *MRI neuroimaging of childhood psychiatric disorders: a selective review.* J Child Psychol Psychiatry, 2000. 41(6): p. 679-94.
44. Castellanos, F.X., et al., *Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder.* Arch Gen Psychiatry, 1996. 53(7): p. 607-16.

4.2

Cerebral ventricular volume and infant temperamental difficulties





Abstract

Background: Numerous studies have provided evidence for subtle deviations in brain morphology in children with psychiatric disorders, but much less is known on the onset and developmental trajectory of these deviations early in life. We aimed to determine whether variances in cerebral ventricular size in fetal and early postnatal life are associated to temperamental difficulties in infancy.

Methods: Within a population-based cohort study, fetal atrial width of the lateral ventricle was measured twice during pregnancy. We used three-dimensional cranial ultrasound to measure cerebral ventricular volume at age 6 weeks. We related size of the cerebral ventricular system to temperamental dimensions at age 3 months using the Mother and Baby Scales and at age 6 months using the Infant Behavior Questionnaire in 1,028 infants.

Results: Fetal lateral ventricular size in mid-pregnancy was not related to temperamental difficulties in infancy, whereas smaller lateral ventricular size in late pregnancy was associated to higher activity at age 6 months. Postnatally, infants with smaller ventricular volumes at age 6 weeks showed higher activity, more anger - irritability, and poorer orienting later in infancy. Children in the lowest quartile of ventricular volume scored on average 0.15 (95% confidence interval: 0.06 – 0.23, p=0.001) points higher, i.e. 23%, on activity level than children in the highest quartile of ventricular volume.

Conclusions: Variations in ventricular size before and shortly after birth are associated with temperamental difficulties. Cautiously interpreting these temperamental difficulties as risk indicators of behavioral problems, the present study suggests that some of the morphologic differences between children with psychopathology and healthy controls may develop very early in life.

Introduction

In the last two decades, numerous studies investigated the associations between anatomical brain structure and child and adolescent psychiatric disorders [1]. Childhood psychiatric disorders, like attention-deficit hyperactivity disorder (ADHD), autism spectrum disorders, and childhood-onset schizophrenia, are hypothesized to be neurodevelopmental in origin [2, 3], although the underlying mechanisms are yet unknown. Subtle changes in the brain may also make individuals more vulnerable for affective disorders [1]. In spite of the large number of studies using neuroimaging modalities in childhood psychiatric disorders, the findings thus far have been inconsistent [1, 4, 5]. This is mainly due to methodological problems, such as heterogeneity of disorders, small sample sizes, different control groups and confounding by pharmacotherapy.

Nonetheless, the studies have shown correlations of brain structural measures with neurodevelopmental disorders on both whole-brain and regional levels. For example, the most consistent structural abnormalities that are described in children with autism spectrum disorders are enlarged total brain size [6-8] and reduced corpus callosum size [9]. Furthermore, abnormalities in the amygdala, hippocampus, and cerebellum have been frequently reported [7]. In children with ADHD, the total brain size is approximately 5% smaller than in age- and sex-matched controls [10]. Distributed subtle anomalies in the brains of children with ADHD have been described in particular in the prefrontal cortices, right caudate, corpus callosum, and cerebellar regions [11-13]. Findings are inconsistent with regard to the ventricular system, which is, alike total brain volume and gray and white matter volume, considered to be a general parameter of brain development [14]. In autism spectrum disorders, one study indicates enlarged lateral ventricles when compared to control children [15], whereas others did not find a statistical difference [16]. Castellanos et al. [10] described a slower increase in lateral ventricular volume in children with attention-deficit hyperactivity disorder compared to control children. Case series in children with schizophrenia reported enlarged ventricles [4, 17, 18], which is also one of the consistent findings in adult patients with schizophrenia [19]. It should be noted however that lateral ventricular volume increase robustly with age in healthy children, which adds to the complexity of interpreting changes in ventricular volume in patient populations [20].

It is unclear whether the subtle changes in the brains of children with psychiatric disorders emerge before the onset of symptoms or are compensatory or adaptive changes of the nervous system. To elucidate whether and when the development of the brain is disturbed in child psychiatric disorders, imaging techniques should be extended to progressively younger age groups of children without disease at time of imaging [21]. Such studies have been conducted in high-risk groups, such as premature born children, and revealed that enlarged ventricles are very common in premature infants and are related to neurodevelopmental outcome in the first years of life [22-25]. Furthermore, mild ventricular enlargement in prenatal life have

been associated with attention-deficit / hyperactivity disorder, autism and learning disorders in a case series of 5 children followed until the age of 4-9 years [26]. However, these studies in high-risk groups cannot distinguish brain damage due to prematurity, pharmacotherapy and mechanical ventilation from subtle changes in the developing brain due to genetic predisposition or environmental factors. In the present study, fetuses and infants were drawn from the general population. Since we aimed to collect data in a large group of mainly healthy children and to keep attrition as low as possible, we used three-dimensional ultrasound as imaging technique. When compared to magnetic resonance imaging, ultrasound has been shown to provide valid volume measurements of the ventricular system [27].

One aspect of social and emotional behavior that can be measured early in life, is temperament. Temperament is theoretically conceptualized as the constitutionally based individual differences in reactivity and self-regulation, which are influenced over time by heredity, maturation, and experience [28]. Numerous studies have argued that temperament plays a role in the etiology and maintenance of behavioral problems in childhood and adolescence [29-31]. Precursors of conduct problems in childhood are susceptibility to anger and hostility in infancy and early childhood [32]. Attention-deficit hyperactivity disorder symptom scores in kindergarten are predicted by irritability, anger, high activity level, as well as difficulties with inhibitory control in the preschool age [33]. Children aged seven years, who had been classified as high reactive, i.e. vigorous motor activity combined with distress and crying, at 4 months, were more likely to have anxious symptoms than those who had been classified as low reactive [34].

We designed this study to examine the association between ventricular size and temperamental difficulties in a sample of 1,028 infants drawn from the general population. Ventricular size was measured twice during pregnancy and postnatally at the age of 6 weeks. We hypothesized that ventricular volumes of children with more difficult temperament at age 3 and 6 months would differ significantly from volumes of children without temperamental problems.

Materials and methods

Participants

This study was conducted within a subgroup of the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood in Rotterdam, the Netherlands, which has been described previously in detail [35, 36].

Detailed prenatal ultrasound assessments were conducted in 1,232 Dutch pregnant women. This subgroup is ethnically homogeneous to exclude confounding or effect modification by ethnicity. No other inclusion or exclusion criteria were defined for participation

in this subgroup. All children were born between February 2003 and August 2005 and form a prenatally enrolled birth cohort that is currently followed until young adulthood. The study was conducted in accordance with the guideline proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

The total of 1,232 mothers delivered 1,244 live births (15 twin pregnancies, 1 intrauterine fetal death, 2 neonatal deaths). Fetal ultrasound of cerebral ventricles was carried out in 692 singleton fetuses during mid-pregnancy and in 1,186 singleton fetuses during late pregnancy. Additional postnatal cranial ultrasounds of sufficient quality were performed in 778 infants. Of all the children with information on cerebral ventricles (n=1,233), data on temperament at age 3 months was available in 939 infants and at age 6 months in 769 infants. Missing data on temperament were mainly due to withdrawn consent for postnatal questionnaires (n=49), logistical problems in sending out the postnatal questionnaires (n=113 for the 3-month-questionnaire, n=273 for the 6-month-questionnaire), or non-response (n=128 for the 3-month-questionnaire, n=138 for the 6-month-questionnaire). Twenty mothers participated with two children (twins or siblings). We randomly excluded one of these siblings to avoid biases due to paired data. A total of 1,028 children (82.6% of 1,244) were included in one or more analyses.

Determinants

Trained sonographers carried out fetal ultrasound examinations at the visits to the research centers in mid- and late pregnancy. The median (95% range) gestational age for the fetal ultrasound examinations in mid- and late pregnancy was 21 (19 – 23) and 30 (28 – 33) weeks. Online measurements used for the present study included atrial width of lateral ventricle and head circumference. Head circumference represents the outer perimeter of the fetal skull, measured in an axial plane. The atrial width of lateral ventricle is the widest diameter of the atrium of one of the lateral ventricles that can be measured in an axial plane. High intra- and inter-observer reproducibility have been reported for head circumference and atrial width of lateral ventricle [37, 38].

We performed postnatal cranial ultrasounds with a commercially available multifrequency electronic transducer usable for three-dimensional volume acquisition (Voluson 730 Expert, GE Healthcare, Waukesha WI, USA). The probe was positioned on the anterior fontanel and a volume box was placed at the level of the foramen of Monro in a symmetrical coronal section. We scanned a pyramid shaped volume of the brain tissue and measured the volume of the lateral ventricular system offline, which was described previously in detail [39]. In short, the volume of the ventricular frontal horns, ventricular body and trigone on both sides was quantified in milliliters. The left ventricle was on average larger (median 0.44, 95% range 0.1 – 1.6

ml) than the right ventricle (median 0.36, 95% range 0.1 – 1.3 ml). Four raters manually traced the left and right ventricles. For reliability analyses, the raters each segmented 20 images twice and images of another 20 children were rated by all four raters. The intra- and inter-observer reliability of the volumetric measurements was very high, partly due to the high variance in ventricular volume within our population. Intra-observer intraclass correlation coefficients varied from 0.989 to 0.993 for the right ventricle and from 0.992 to 0.997 for the left ventricle. Inter-observer intraclass correlation coefficients were 0.950 for the right ventricle and 0.981 for the left ventricle.

Infant temperament

Mothers completed the Mother and Baby Scales (MABS) [40, 41] for their child at age 3 months and the Infant Behavior Questionnaire – Revised (IBQ-R) [28] at age 6 months. We used the two infant scales of the MABS; infant irritability and alertness. The irritability scale contains 15 items, e.g. 'My baby has fussed before settling down', and the alertness scale consists of 8 items, e.g. 'When I talk to my baby, s/he seems to take notice'. Each item was rated on 6-point rating scales ranging from 0=not at all to 5=very much/often. Total scores ranged from 0-75 for irritability and from 0-40 for alertness. A higher score on the scale irritability indicates more difficult behavior in that dimension, whereas a higher score on the scale alertness indicates less difficult behavior. Internal consistencies were 0.75 for irritability and 0.64 for alertness.

We chose six out of the original 14 scales of the IBQ-R, because the complete instrument of 191 items was not feasible within our multidisciplinary study with numerous assessments. The six scales included activity level, distress to limitations, fear, duration of orienting, falling reactivity, and sadness, and were judged of particular importance from a clinical perspective for the most prevalent behavioral disorders in childhood. Activity level relates to gross motor activity and squirming. Distress to limitations refers to negative emotionality and reaction to frustrating situations. Fear includes rejection of new objects and persons. Duration of orienting comprises items on attention and distractibility. Falling reactivity refers to rate of recovery from peak distress or frustration. Finally, questions about sadness relate to lowered mood due to personal suffering or object loss. The original 7-point scale was adapted to a 3-point scale (0=never present, 1=sometimes present and 2=often present). We adapted the original 7-point scale to a 3-point scale (0=never present, 1=sometimes present and 2=often present), since the participants of a pilot study seldom used the extreme positions of scales. Moreover, when a large number of individual items are designed to be summed to create a scale score, it is likely that reducing the number of levels to three will not result in significant loss of information [42]. Higher scores on the scales, besides on falling reactivity, indicate more difficult behavior. The scores for each scale were calculated by dividing the sum of the items by the number of completed items. According to the concept that traits can be consolidated into a small number of dimensions [30], we grouped distress to limitations, recovery from

distress, and irritability into irritability/anger, fear and sadness into behavioral inhibition, and duration of orienting and alertness-responsiveness into orienting [43]. Activity level does not fall into one of these categories as it represents the dimension approach / positive affect.

Covariates

We considered socio-economic status related variables and psychosocial stressors, as well as obstetric and neonatal variables, as possible confounders. Furthermore, we adjusted our associations for other known determinants of infant temperament, such as infant gender, infant age, maternal psychopathology, and maternal self-esteem. Date of birth, birth weight and gender of the infant were obtained from community midwife and hospital registries at birth. We established gestational age by using the fetal ultrasound examinations within the Generation R Study. During the postnatal visit at age 6 weeks, we measured the fronto-occipital head circumference (cm). Maternal age, maternal educational level, and parity were determined at enrolment. To assess maternal psychopathology in mid-pregnancy and 3 months postpartum, we used the Brief Symptom Inventory (BSI) [44, 45]. We used the 12-item subscale General Functioning of the McMaster Family Assessment Device [46] to determine family functioning. Long-lasting difficulties were measured with a 12 item-checklist [47]. Maternal self-esteem was assessed in late pregnancy, using The Rosenberg Self-Esteem Scale [48]. Community midwife and hospital registries at birth yielded information on mode of delivery and Apgar scores. Mode of delivery was classified as 'caesarean section', 'spontaneous vaginal delivery' and 'instrumental vaginal delivery'.

Statistical analyses

To examine whether non-response was selective, we compared core data of children with information on ventricular volume and temperament to eligible children within the subgroup without information on ventricular volume or on temperament (n=216; 1,244 -/- 1,028 children).

The associations between ventricular size and temperamental dimensions were analyzed using multivariable linear regression models. First, we log-transformed postnatal ventricular volume to satisfy the assumption of normality. The temperamental dimensions fear, falling reactivity, and alertness, were log- or square transformed to achieve normality or constant variance. Next, we derived standard deviation scores for both determinants and outcomes to make regression coefficients for transformed and non-transformed outcomes comparable. Since the regression coefficients are not straightforward to interpret, we additionally defined quartiles of ventricular volume and used analyses of covariance (ANCOVA) to compare mean scores of non-transformed scales between quartiles. All associations were controlled for head circumference at time of measurement of ventricular size, since information about ventricular

size is difficult to interpret without placing it in the context of overall head size. The other covariates were selected as a result of exploratory analyses, and were included in the analyses if effect estimates of ventricular size changed meaningfully (defined as more than 5%). Moreover, we excluded collinear covariates to avoid instability of the models. For example maternal self-esteem, family functioning, and long-lasting difficulties were highly correlated to maternal psychopathology, and had no additional confounding effect on the association between ventricular size and infant temperament. Other variables like maternal smoking, maternal alcohol consumption, single parenthood, financial difficulties, obstetric or perinatal complications (Apgar score, mode of delivery, birth weight, gestational diabetes, maternal hypertension, pre-eclampsia), child anthropometrics, and child birth order did not confound the association between ventricular volume and infant temperament, and were therefore not included in the final models. We carried out a post-hoc Bonferroni adjustment to correct for the multiple hypotheses with regard to different temperamental scales per end-point in age. When determinants and endpoints are highly correlated, the conservatism of the Bonferroni method increases and the power of detecting association effects is reduced. Since the ventricular measures were highly correlated, we chose to correct for two hypotheses at age 3 months and for six hypotheses at age 6 months. We tested whether interaction terms of ventricular size and gender were significant at $\alpha = 0.15$. Measures of association are presented with their 95% confidence intervals (CI). Statistical analyses were carried out using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

Response analyses

Analyses of missing data showed that children without data on determinants or outcome had on average 119 (95% confidence interval (CI): 16; 221) grams lower birth weight. Their mothers were 1.3 (95% CI: 0.5; 2.1) years younger and were significantly lower educated (% higher education 51.4% vs. 64.9%, $\chi^2 = 22.3$, df = 2, $p < 0.001$). Children with missing data on determinants or outcome did not differ from children included in the analyses in median gestational duration or parental psychopathology. Furthermore, ventricular sizes at all three time-points did not differ between children with and children without information on temperament.

Results

Table 1 presents the characteristics of participating children and their parents. 50.2% of this sample was male. Birth weight and head circumference at gestational age 20 and 30 weeks and age 6 weeks, were larger in boys than in girls. Median gestational duration was 40.1 weeks, ranging from 28 to 43 weeks. In total, 40 children (3.9%) were born before 37 weeks.

Table 1. Subject characteristics

	Boys (n = 516)	Girls (n=512)
Child characteristics		
Gestational duration (weeks)	40.3 (35.9 – 42.4)	40.1 (36.1 – 42.4)
Birth weight (grams)	3567 (539)	3478 (540)
Apgar-score 1 minute after birth	9 (5 – 10)	9 (6 – 10)
Age at time of postnatal visit (weeks)	6.8 (4.4 – 12.3)	6.4 (4.4 – 12.0)
Head circumference at postnatal visit (mm)	390 (14.2)	383 (14.6)
Parental characteristics		
Maternal age (years)	31.6 (4.0)	32.0 (3.8)
Maternal educational level (% high)	63.8	66.1
Parity (% nulli)	63.1	61.8
Mode of delivery (%)		
Spontaneous vaginal	65.9	68.2
Instrumented vaginal	20.2	15.6
Caesarean section	13.9	16.2
Maternal psychopathology (total score)		
In mid-pregnancy	0.1 (0.0 – 0.8)	0.1 (0.0 – 0.7)
3 months postpartum	0.1 (0.0 – 0.9)	0.1 (0.0 – 0.8)
Family functioning, according to mother (% pathological)	2.7	4.5
Long-lasting difficulties mother (score)	1.0 (0 – 8)	1.0 (0 – 9)
Maternal self-esteem (score)	4.53 (2.9 – 5.0)	4.53 (3.1 – 5.0)
Main determinants		
Atrial width of lateral ventricle in mid-pregnancy (mm)	5.9 (1.1)	5.6 (1.2)
Atrial width of lateral ventricle in late pregnancy (mm)	5.4 (1.8)	5.1 (1.7)
Ventricular volume (ml)	0.84 (0.15 – 3.24)	0.76 (0.14 – 2.65)

Values are means (SD) for continuous normally distributed variables, medians (95% range) for continuous non-normally distributed variables and percentages for categorical variables.

Mean maternal age at intake in pregnancy was 31.8 years, ranging from 18 to 46 years. Of all participating children, 62% was the first-born child within the family. Mean (and standard deviations) of the ventricular measurements were 5.8 (1.2) mm for the atrial width of lateral ventricle in mid-pregnancy, 5.3 (1.7) mm for the atrial width of lateral ventricle in late pregnancy, and –0.26 (0.7) for the log-transformed postnatal ventricular volume.

Table 2 presents the associations between ventricular size and temperament at the age of 3 months. Ventricular size in mid- and late pregnancy was not related to temperament in 3-month-olds. However, smaller ventricular volumes measured in postnatal life was associated with orienting at the age of 3 months. Per standard deviation smaller ventricular volume, the score of infant alertness was 0.10 standard deviation lower. This association remained statistically significant after Bonferroni's correction. There were no significant gender differences. Head circumference in late pregnancy was related to infant alertness only (β: -0.01; 95% confidence interval: -0.01; -0.00, p = 0.006). However, the associations between ventricular size and infant temperament could not be explained by head circumference.

Furthermore, we related ventricular size at three time-points to infant temperament at the age of 6 months (table 3). Again, ventricular size in mid-pregnancy was not associated to infant

Table 2. Associations between ventricular size and infant temperament at age 3 months

Ventricular assessment	Irritability / Anger Unsettled – Irregularity (SD) †				Orienting Alertness – Responsiveness (SD) † ‡			
	β (95% CI)	p	R^2	F	β (95% CI)	p	R^2	F
Prenatal:								
Lateral ventricle in mid-pregnancy (per SD)	-0.01 (-0.09; 0.07)	0.8	0.29	5.0	-0.04 (-0.12; 0.04)	0.3	0.22	2.7
Lateral ventricle in late pregnancy (per SD)	-0.02 (-0.09; 0.04)	0.5	0.30	9.4	0.03 (-0.03; 0.10)	0.3	0.27	7.5
Postnatal:								
Lateral ventricles at age 6 weeks (per SD) #	-0.08 (-0.16;-0.00)	0.05	0.30	6.8	0.10 (0.01; 0.18)	0.02	0.28	5.5

Values are regression coefficients (95% confidence intervals) from multivariable linear regression and reflect the difference in z-score of temperamental score per standard deviation atrial width of lateral ventricle or postnatal ventricular volume. Models are adjusted for age, gender, gestational duration, head circumference at time of measurement ventricle, maternal age, maternal educational level (in three categories), and maternal psychopathology. The number of subjects varies per measurement of ventricular size (in total 912): in mid-pregnancy n=504, in late pregnancy n=894, postnatally n=580.

SD = standard deviation

CI = confidence interval

P = p-value of ventricular size

F= F-statistic with 9 degrees of freedom in the numerator. Degrees of freedom in the denominator were 497, 882, and 570 for irritability / anger, and 498, 885, and 571 for orienting. These F-statistics and degrees of freedom apply to the complete models, including covariates.

R^2 = R-squared, coefficient of multiple determination. This coefficient applies to the complete model.

† Higher scores on Alertness – Responsiveness indicate less difficult behavior, whereas a higher score on Unsettled-Irregularity indicate more difficult behavior.

‡ Scores on this scale were square transformed to satisfy the assumption of linearity.

Ventricular volumes were log-transformed to normalize the distribution.

temperament. In late pregnancy, smaller ventricular size only predicted higher activity level. Postnatally, smaller ventricular volume was associated to both scales of anger / irritability and to higher activity. Per standard deviation smaller ventricular volume at age 6 weeks, activity level at age 6 months was 0.18 standard deviation higher, and infant distress to limitations was 0.14 standard deviation higher, whereas rate of recovery from distress was 0.12 standard deviation lower. We found no significant associations of postnatal ventricular volumes with fear, sadness, or duration of orienting. If Bonferroni's correction was used to establish more conservative alphas ($\alpha = 0.05 / 6 = 0.0083$), the associations of postnatal ventricular volume with activity level and distress to limitations remained statistically significant. None of the interaction terms of ventricular size and gender was significant at $\alpha = 0.15$. Head circumference at age 6 weeks was related to distress to limitations only (β: 0.06; 95% confidence interval: 0.00; 0.12, $p = 0.04$). However, head circumference did not account for the association between ventricular size and infant temperament. Moreover, exclusion of the premature born children (gestational age at birth < 37 weeks, n = 40) did not change our results.

Table 3. Associations between ventricular size and infant temperament at age 6 months

Ventricular assessment	Irritability / Anger								Activity level			
	Distress to limitations				Falling reactivity † ‡				Activity level			
	β (95% CI)	p	R^2	F	β (95% CI)	p	R^2	F	β (95% CI)	p	R^2	F
Prenatal:												
Lateral ventricle in mid-pregnancy (per SD)	-0.03 (-0.12;0.06)	0.5	0.21	2.7	0.04 (-0.05; 0.13)	0.4	0.15	1.3	-0.07 (-0.16;0.02)	0.1	0.28	4.8
Lateral ventricle in late pregnancy (per SD)	-0.06 (-0.13;0.02)	0.1	0.21	3.6	-0.01 (-0.09;0.07)	0.8	0.13	1.3	-0.09 (-0.17;-0.02)	0.02	0.30	7.6
Postnatal:												
Lateral ventricles at age 6 weeks (per SD) #	-0.14 (-0.24;-0.05)	0.004	0.26	3.4	0.12 (0.01; 0.22)	0.03	0.16	1.3	-0.18 (-0.27;-0.08)	0.0004	0.30	4.9
	Behavioral inhibition								Orienting			
	Fear				Sadness §				Duration of orienting			
	β (95% CI)	p	R^2	F	β (95% CI)	p	R^2	F	β (95% CI)	p	R^2	F
Prenatal:												
Lateral ventricle in mid-pregnancy (per SD)	0.04 (-0.05;0.13)	0.4	0.24	3.5	-0.05 (-0.14;0.04)	0.3	0.16	1.5	0.05 (-0.04; 0.14)	0.3	0.21	2.5
Lateral ventricle in late pregnancy (per SD)	-0.06 (-0.14;0.02)	0.1	0.24	4.7	-0.06 (-0.14;0.02)	0.1	0.17	2.3	0.06 (-0.01; 0.14)	0.1	0.20	3.2
Postnatal:												
Lateral ventricles at age 6 weeks (per SD) #	-0.07 (-0.17;0.03)	0.2	0.24	3.0	-0.05 (-0.15;0.05)	0.3	0.19	1.8	0.01 (-0.09; 0.11)	0.8	0.17	1.4

Values are regression coefficients (95% confidence intervals) from multivariable linear regression and reflect the difference in z-score of temperamental score per standard deviation of atrial width of lateral ventricle or postnatal ventricular volume. Models are adjusted for age, gender, gestational duration, head circumference at time of measurement ventricle, maternal age, maternal educational level, and maternal psychopathology. The number of subjects per analysis varies per measurement of ventricular size (in total 745): in mid-pregnancy n=516, in late pregnancy n=716, postnatally n=441.

CI = confidence interval

SD = standard deviation

P = p-value of ventricular size

F= F-statistic with 9 degrees of freedom in the numerator. Degrees of freedom in the denominator were 505, 707, and 433 for distress to limitations, 497, 696, and 425 for falling reactivity, and 504, 707, and 431 for activity level. For fear, the degrees of freedom in the denominator were 511, 711, and 436, for sadness 509, 708, and 436, and for duration of orienting 502, 705, and 431. These F-statistics and degrees of freedom apply to the complete models, including covariates.

R^2 = R-squared, coefficient of multiple determination. This coefficient applies to the complete model.

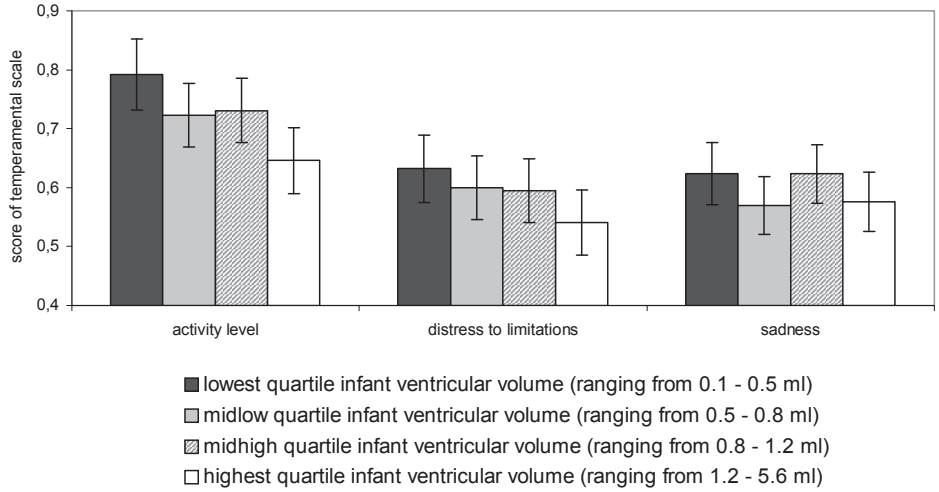
† Higher scores on Falling Reactivity indicate less difficult behavior, whereas higher scores on the other dimensions indicate more difficult behavior.

‡ Scores on this scale were square transformed to satisfy the assumption of linearity.

§ Scores on this scale were square root transformed to satisfy the assumption of linearity.

Ventricular volumes were log-transformed to normalize the distribution.

Figure 1. Mean scores of activity level, distress to limitations, and sadness, in quartiles of postnatal ventricular volume



Values are estimated means and 95% confidence intervals for scores on activity level, distress to limitations, and sadness (scales ranging from 0 to 2), in quartiles of postnatal ventricular volume, adjusted for age, gender, gestational duration, head circumference at time of three-dimensional postnatal ultrasound, maternal age, maternal educational level, and maternal psychopathology.

To illustrate the relation, we present in figure 1 the adjusted mean scores on activity level, distress to limitations, and sadness, per quartile of ventricular volume. Children in the lowest quartile of ventricular volume scored on average 0.15 (95% CI: 0.06; 0.23, p=0.001) points higher, i.e. 23%, on activity level compared to children in the highest quartile of ventricular volume. The mean score on distress to limitations for children in the lowest quartile was 0.09 (95% CI: 0.01; 0.17, p=0.03) points higher, i.e. 17%, than the mean score for children in the highest quartile. The non-significant difference in score on sadness between children in the lowest and the highest quartile of ventricular volume was 0.05 (95% CI: -0.03; 0.12, p=0.21) points.

Discussion

This large population-based study showed that variation in brain morphology in fetal and early postnatal life is associated to temperamental difficulties at age 3 and 6 months. Infants with a smaller size of the cerebral ventricular system were more likely to have temperamental problems. Infant ventricular volume was related to infant alertness at the age of 3 months. Furthermore, smaller ventricular volume in infancy was associated with more anger / irritability and higher activity at age 6 months. The size of the prenatal cerebral ventricular system was less strongly associated to infant temperament. Smaller ventricles in late pregnancy were

only related to higher activity at age 6 months, whereas ventricular size in mid-pregnancy did not predict temperamental problems.

To our knowledge, this study is the first attempt to relate brain morphology to behavior within the normal population at such a young age. Early behavioral and emotional characteristics of (very) young children, or temperamental features, are thought to have a constitutional basis and form a relatively enduring make-up of the individual [49]. Temperamental traits have been frequently related to behavior and psychopathology in childhood and later in life. Higher infant activity level is related to outgoing activity and positive anticipation as well as impulsivity, anger/frustration, and lower inhibitory control. Irritability and frustration in infancy predicted later externalizing negative affect [43] as well as oppositional and conduct problems [30]. Moreover, hostile and aggressive behavior, anger, and difficulties with inhibitory control are precursors of attention-deficit hyperactivity disorder [33]. This suggests that the smaller ventricles observed in our study indicate a higher risk to develop externalizing psychopathology, including attention-deficit hyperactivity disorder.

Most reports on ventricular volume and childhood psychiatric disorders described larger ventricles in childhood-onset schizophrenia, autism, depression, and eating disorders [4]. However, interpreting changes in ventricular volume in patient populations is complex, since studies in normally developing children and adolescence reported robust increases of lateral ventricular volume with age [20]. The interpretation of this complexity would benefit from prospective studies in populations that are epidemiologically ascertained, which might help to identify the longitudinal trajectories of illness-related abnormalities in the brain [21]. Our results indicate that smaller ventricular volume in infancy may increase the risk of behavioral problems, which is in line with the finding of Castellanos et al [10], who described a diminished age-related increase in lateral ventricular volume from age 5 – 18 years in children with attention-deficit hyperactivity disorder compared to controls. Since knowledge on the maturational progress of the ventricular system is limited, we can only speculate about the underlying mechanism of the association between smaller ventricular volumes and temperamental difficulties. Possibly, smaller ventricular volumes are the effect of delayed or reduced apoptosis of overproduced neurons, which is one of the normally occurring regressive phenomena during development of the nervous system [50, 51]. Another possible mechanism that explains the association between small ventricles and temperamental difficulties might be the reduced secretion of cerebrospinal fluid, which leads to a loss of the essential expanding pressure within the ventricular system that might determine brain morphology during its development [52].

Small changes in brain development during prenatal and early postnatal life may be the consequence of several adverse environmental factors in utero. First, placental insufficiency may lead to chronic fetal hypoxia and shortage of other nutrients such as glucose and amino acids [53]. This not only leads to intrauterine growth restriction, but may also lead to altered brain structure and abnormalities in brain function [54]. Second, maternal smoking during

pregnancy, as well as maternal gestational stress, have been shown to have direct negative effects on the developing brain [55, 56]. These environmental factors have also been related to behavioral problems later in life.

Although the effect sizes were small, the associations between a smaller ventricular volume and temperamental problems were consistent for both 3 months and 6 months of age and pronounced for problems related to externalizing behavior. However, several other methodological considerations need to be discussed. First, selective participation of healthier, higher educated, and older mothers may have impeded the detection of modest associations. We had no indication that ventricular size was different in children of younger and lower educated women, but the selective attrition of mothers with prenatally and perinatally complicated pregnancies could lead to bias. Children of these mothers might have had larger postnatal ventricular size due to white matter damage or intraventricular hemorrhage and, in consequence, more behavioral problems. Therefore, we should be cautious in generalizing our findings to clinical populations of e.g. premature born children. Second, the lower resolution of ultrasound in comparison with magnetic resonance imaging necessitates manual tracing of ventricles and precludes tracing of other brain regions. However, we showed a rather high reproducibility for the ventricular measurements. Third, using a maternal report of temperamental traits might introduce reporter bias, since these questionnaires reflect not only a report of actual child behavior but also a component colored by parental characteristics [57]. However, we assume this type of misclassification is non-differential, since mothers were blinded to their infant's brain morphology. Furthermore, we adjusted our associations for maternal psychopathology, which did not fully explain the associations between ventricular size and infant temperament. Although Seifer et al. suggested that laboratory or home observations reliably and validly measure infant temperament and poorly corresponded with parent reports [57], maternal report of temperament takes advantage of the primary caregivers' opportunity to observe her infant across different contexts [28]. Fourth, we need to mention the limited categories in our adapted version of the IBQ, which may have reduced the variation of temperamental scores. This would decrease the chance of detecting statistically significant effects. Fifth, our study cannot provide direct information on the developing trajectory of lateral ventricles throughout prenatal and early postnatal life, since the two-dimensional measurements of the atrium during pregnancy and the three-dimensional measurement of the frontal horns and ventricular body after birth are not equivalent. We previously reported that a smaller size and a decreasing growth pattern of the lateral ventricle atrial width in pregnancy were predictive of smaller ventricular volume in infancy [39]. The present study, however, suggests that postnatal measurement of ventricular size is superior over prenatal ventricle measurement in predicting infant temperament. Finally, due to the close temporal proximity of measurement of the main determinant postnatal ventricular volume and assessment of temperamental outcome, inferences about cause and effect have to be made with caution.

In conclusion, our study provides direct evidence that cerebral ventricular volume in infancy is related to temperamental difficulties. This suggests that the differences in brain morphology between children with specific psychiatric disorders and normal controls could develop very early in life. While it may be premature to speculate about clinical implications such as cranial ultrasound screening, our results suggest that it is worthwhile to follow-up chance findings of very small ventricular volume in full-term infants, particularly when accompanied by maternal concerns about her child's temperamental difficulties. Future studies should address the development of brain structure from young infancy onwards in relation to emotional and behavioral problems in childhood and adolescence. Ideally, this should be investigated in large prospective samples from the general population or in high-risk groups.

References

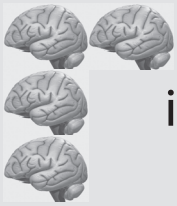
1. Giedd, J.N., et al., *Anatomic brain imaging studies of normal and abnormal brain development in children and adolescents*, in *Developmental Psychopathology (2nd ed.).: Developmental Neuroscience*, D. Cicchetti and D. Cohen, Editors. 2006, Wiley: New York. p. 127-196.
2. Filipek, P.A., *Neuroimaging in the developmental disorders: the state of the science.* J Child Psychol Psychiatry, 1999. 40(1): p. 113-28.
3. Bradshaw, J.L. and D.M. Sheppard, *The neurodevelopmental frontostriatal disorders: evolutionary adaptiveness and anomalous lateralization.* Brain Lang, 2000. 73(2): p. 297-320.
4. Hendren, R.L., I. De Backer, and G.J. Pandina, *Review of neuroimaging studies of child and adolescent psychiatric disorders from the past 10 years.* J Am Acad Child Adolesc Psychiatry, 2000. 39(7): p. 815-28.
5. Eliez, S. and A.L. Reiss, *MRI neuroimaging of childhood psychiatric disorders: a selective review.* J Child Psychol Psychiatry, 2000. 41(6): p. 679-94.
6. Redcay, E. and E. Courchesne, *When is the brain enlarged in autism? A meta-analysis of all brain size reports.* Biol Psychiatry, 2005. 58(1): p. 1-9.
7. DiCicco-Bloom, E., et al., *The developmental neurobiology of autism spectrum disorder.* J Neurosci, 2006. 26(26): p. 6897-906.
8. Courchesne, E., et al., *Autism at the beginning: microstructural and growth abnormalities underlying the cognitive and behavioral phenotype of autism.* Dev Psychopathol, 2005. 17(3): p. 577-97.
9. Waiter, G.D., et al., *Structural white matter deficits in high-functioning individuals with autistic spectrum disorder: a voxel-based investigation.* Neuroimage, 2005. 24(2): p. 455-61.
10. Castellanos, F.X., et al., *Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder.* Arch Gen Psychiatry, 1996. 53(7): p. 607-16.
11. Krain, A.L. and F.X. Castellanos, *Brain development and ADHD.* Clin Psychol Rev, 2006. 26(4): p. 433-44.
12. Valera, E.M., et al., *Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder.* Biol Psychiatry, 2007. 61(12): p. 1361-9.
13. Seidman, L.J., E.M. Valera, and N. Makris, *Structural brain imaging of attention-deficit/hyperactivity disorder.* Biol Psychiatry, 2005. 57(11): p. 1263-72.
14. Gilmore, J.H., et al., *Mild ventriculomegaly detected in utero with ultrasound: clinical associations and implications for schizophrenia.* Schizophr Res, 1998. 33(3): p. 133-40.
15. Piven, J., et al., *An MRI study of brain size in autism.* Am J Psychiatry, 1995. 152(8): p. 1145-9.
16. Hardan, A.Y., et al., *Brain volume in autism.* J Child Neurol, 2001. 16(6): p. 421-4.
17. Woody, R.C., et al., *CT scan and MRI findings in a child with schizophrenia.* J Child Neurol, 1987. 2(2): p. 105-10.
18. Schulz, S.C., et al., *Ventricular enlargement in teenage patients with schizophrenia spectrum disorder.* Am J Psychiatry, 1983. 140(12): p. 1592-5.
19. Wright, I.C., et al., *Meta-analysis of regional brain volumes in schizophrenia.* Am J Psychiatry, 2000. 157(1): p. 16-25.
20. Lenroot, R.K. and J.N. Giedd, *Brain development in children and adolescents: insights from anatomical magnetic resonance imaging.* Neurosci Biobehav Rev, 2006. 30(6): p. 718-29.
21. Peterson, B.S., *Conceptual, methodological, and statistical challenges in brain imaging studies of developmentally based psychopathologies.* Dev Psychopathol, 2003. 15(3): p. 811-32.
22. Thompson, D.K., et al., *Perinatal risk factors altering regional brain structure in the preterm infant.* Brain, 2007. 130(Pt 3): p. 667-77.
23. Inder, T.E., et al., *Abnormal cerebral structure is present at term in premature infants.* Pediatrics, 2005. 115(2): p. 286-94.
24. Miller, S.P., et al., *Early brain injury in premature newborns detected with magnetic resonance imaging is associated with adverse early neurodevelopmental outcome.* J Pediatr, 2005. 147(5): p. 609-16.

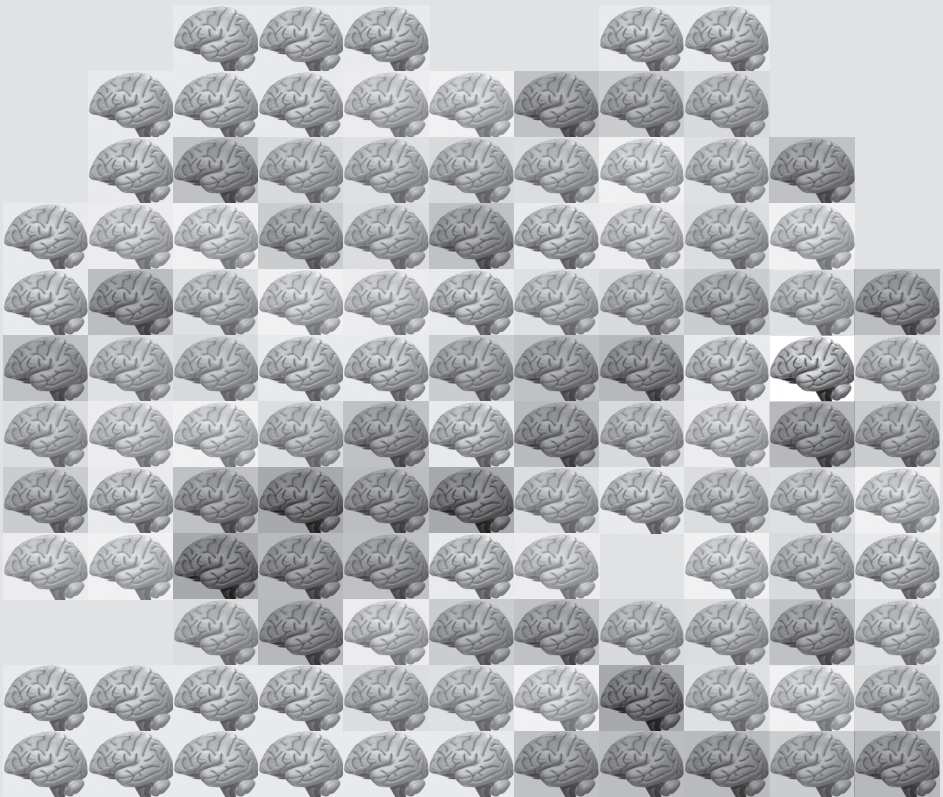
25. Dyet, L.E., et al., *Natural history of brain lesions in extremely preterm infants studied with serial magnetic resonance imaging from birth and neurodevelopmental assessment.* Pediatrics, 2006. 118(2): p. 536-48.
26. Gilmore, J.H., et al., *Outcome in children with fetal mild ventriculomegaly: a case series.* Schizophr Res, 2001. 48(2-3): p. 219-26.
27. Gilmore, J.H., et al., *Infant cerebral ventricle volume: a comparison of 3-D ultrasound and magnetic resonance imaging.* Ultrasound Med Biol, 2001. 27(8): p. 1143-6.
28. Gartstein, M.A. and M.K. Rothbart, *Studying infant temperament via the Revised Infant Behavior Questionnaire.* Infant Behavior & Development, 2003. 26: p. 64 - 86.
29. Rothbart, M.K. and M.I. Posner, *Temperament, attention, and developmental psychopathology*, in *Developmental Psychopathology (2nd ed.).: Developmental Neuroscience*, D. Cicchetti and D. Cohen, Editors. 2006, Wiley: New York. p. 465 - 501.
30. Nigg, J.T., *Temperament and developmental psychopathology.* J Child Psychol Psychiatry, 2006. 47(3-4): p. 395-422.
31. Muris, P. and T.H. Ollendick, *The role of temperament in the etiology of child psychopathology.* Clin Child Fam Psychol Rev, 2005. 8(4): p. 271-89.
32. Frick, P.J. and A.S. Morris, *Temperament and developmental pathways to conduct problems.* J Clin Child Adolesc Psychol, 2004. 33(1): p. 54-68.
33. Nigg, J.T., H.H. Goldsmith, and J. Sachek, *Temperament and attention deficit hyperactivity disorder: the development of a multiple pathway model.* J Clin Child Adolesc Psychol, 2004. 33(1): p. 42-53.
34. Kagan, J., et al., *Infant temperament and anxious symptoms in school age children.* Dev Psychopathol, 1999. 11(2): p. 209-24.
35. Hofman, A., et al., *Growth, development and health from early fetal life until young adulthood: the Generation R Study.* Paediatr Perinat Epidemiol, 2004. 18(1): p. 61-72.
36. Jaddoe, V.W., et al., *The Generation R Study: Design and cohort profile.* Eur J Epidemiol, 2006. 21(6): p. 475-84.
37. Perni, S.C., et al., *Intraobserver and interobserver reproducibility of fetal biometry.* Ultrasound Obstet Gynecol, 2004. 24(6): p. 654-8.
38. Cardoza, J.D., R.B. Goldstein, and R.A. Filly, *Exclusion of fetal ventriculomegaly with a single measurement: the width of the lateral ventricular atrium.* Radiology, 1988. 169(3): p. 711-4.
39. Roza, S.J., et al., *Foetal growth determines cerebral ventricular volume in infants. The Generation R Study.* NeuroImage, 2007. doi: 10.1016/j.neuroimage.2007.11.004.
40. St James-Roberts, I. and D. Wolke, *Convergences and discrepancies, among mothers' and professionals' assessments of difficult neonatal behaviour.* J Child Psychol Psychiatry, 1988. 29(1): p. 21-42.
41. Wolke, D. and I. St James-Roberts, *Multi-method measurement of the early parent-infant system with easy and difficult newborns*, in *Psychobiology and early development.*, H. Rauh and H.C. Steinhausen, Editors. 1987, Oxford: North-Holland. p. 49-70.
42. Streiner, D.L. and G.R. Norman, *Health Measurement Scales: a practical guide to their development and use*. 3rd ed. 2003, New York: Oxford University Press.
43. Rothbart, M.K., S.A. Ahadi, and D.E. Evans, *Temperament and personality: origins and outcomes.* J Pers Soc Psychol, 2000. 78(1): p. 122-35.
44. de Beurs, E., *Brief Symptom Inventory, handleiding.* 2004: Leiden, the Netherlands.
45. Derogatis, L.R. and N. Melisaratos, *The Brief Symptom Inventory: an introductory report.* Psychol Med, 1983. 13(3): p. 595-605.
46. Byles, J., et al., *Ontario Child Health Study: reliability and validity of the general functioning subscale of the McMaster Family Assessment Device.* Fam Process, 1988. 27(1): p. 97-104.
47. Hendriks, A.A.J., J. Ormel, and G. van de Willige, *Long lasting difficulties measured with a self assessment questionnaire and semi structured interview: a theoretical and empirical comparison [in Dutch].* Gedrag en Gezondheid, 1990. 18: p. 273-283.
48. Rosenberg, M., *Conceiving the Self*. 1979, New York: Basic Books.

49. Whittle, S., et al., *The neurobiological basis of temperament: towards a better understanding of psychopathology.* Neurosci Biobehav Rev, 2006. 30(4): p. 511-25.
50. Chugani, H.T., *Biological basis of emotions: brain systems and brain development.* Pediatrics, 1998. 102(5 Suppl E): p. 1225-9.
51. Webb, S.J., C.S. Monk, and C.A. Nelson, *Mechanisms of postnatal neurobiological development: implications for human development.* Dev Neuropsychol, 2001. 19(2): p. 147-71.
52. Dziegielewska, K.M., et al., *Development of the choroid plexus.* Microsc Res Tech, 2001. 52(1): p. 5-20.
53. Baschat, A.A., *Fetal responses to placental insufficiency: an update.* Bjog, 2004. 111(10): p. 1031-41.
54. Rehn, A.E., et al., *An animal model of chronic placental insufficiency: relevance to neurodevelopmental disorders including schizophrenia.* Neuroscience, 2004. 129(2): p. 381-91.
55. Ernst, M., E.T. Moolchan, and M.L. Robinson, *Behavioral and neural consequences of prenatal exposure to nicotine.* J Am Acad Child Adolesc Psychiatry, 2001. 40(6): p. 630-41.
56. Davis, E.P., et al., *Prenatal exposure to maternal depression and cortisol influences infant temperament.* J Am Acad Child Adolesc Psychiatry, 2007. 46(6): p. 737-46.
57. Seifer, R., et al., *Infant temperament measured by multiple observations and mother report.* Child Dev, 1994. 65(5): p. 1478-90.

4.3

Cerebral ventricular volume and infant neuromotor development





Abstract

Objective: To investigate the association between lateral ventricular volume and neuromotor development prospectively in normal infants.

Methods: Within the Generation R Study, a population-based cohort, 775 infants underwent three-dimensional cranial ultrasound to determine lateral ventricular volume at the age of 6 weeks. Age-appropriate neurodevelopmental assessment by research assistants at age 6 weeks, 6 months, and 14 months, was used to define optimal and non-optimal muscle tone. Attainment of gross and fine motor milestones was assessed by parent report.

Results: There was a curvilinear relation between volume of the cerebral ventricular system in young infancy and lower muscle tone (adjusted odds ratio of highest quartile versus mid-low quartile 2.2 (95% confidence interval: 1.5 to 3.3, $p<0.001$) and adjusted odds ratio of lowest quintile versus mid-low quintile 1.5 (1.0 to 2.2, $p=0.05$)). Likewise, the association between ventricular volume and fine motor milestone attainment was U-shaped (p-value of quadratic term: 0.002). Ventricular volume was linearly related to attainment of gross motor milestones. Children in the highest quartile of ventricular volume had on average 0.7 (0.2 to 1.1, $p<0.001$) month delay in gross motor development compared to children in the lowest quartile of ventricular volume.

Interpretation: Our findings provide evidence from a non-clinical population that early non-optimal motor development has neurostructural correlates. This study suggests that both extremes of ventricular volume may point to an immature brain with consequences for motor development.

Introduction

Many developmental disabilities, like cerebral palsy, result from brain lesions that originate in prenatal and early postnatal life. Recently, it has been suggested that subtle, 'transient' neuromotor dysfunction in infancy can also be due to central nervous system abnormalities that may later surface in the form of cognitive and behavioral problems [1]. Research in preterms has indicated that small alterations in muscle tone, muscle power or quality of movements in infancy are, despite their limited prognostic importance for major disabilities [2, 3], markers for developmental delay [4], minor neurological dysfunction [5], coordination problems [6], attention problems, and aggressive behaviour [7] in childhood. Longitudinal studies in large general population samples provided evidence for a relationship of early motor developmental delay with adult intellectual function [8, 9] and schizophreniform disorder in adulthood [10].

Studies in premature infants showed that structural alterations in the brain, even if not accompanied by major principal lesions, are related to developmental disabilities [11-13]. Prominently increased lateral ventricular or cerebrospinal fluid volume for example, was related to developmental disabilities in the first year of life [12].

Very little is known on the variation in cerebral structural volume in the general population and its consequence for neuromotor development. We related cerebral ventricular volume, considered to be a general parameter of brain development [14], in 775 infants aged 6 weeks to neuromotor outcomes at different time-points. We addressed the research question whether variation in ventricular volume is related to subtle variation in muscle tone and the attainment of motor milestones. In line with earlier research in preterms, we hypothesized that larger ventricular volume predicts worse motor outcome in healthy term infants.

Methods

Setting and study population

This study was conducted within the Generation R Focus Study, a population-based cohort study from fetal life until young adulthood, which has been described previously [15]. This subgroup of the Generation R Study is ethnically homogeneous to exclude confounding or effect modification by ethnicity. All children were born between February 2003 and August 2005 and form a prenatally enrolled birth cohort.

This study was conducted in accordance with the guideline proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all mothers of participating children.

A total of 778 infants underwent three-dimensional cranial ultrasound at the age of 6 weeks. A neurodevelopmental assessment was available in 727 infants at age 6 weeks, in 672 infants at age 6 months, and in 593 infants at age 14 months. Parent reports on achieved motor milestones were available for 386 infants at age 6 months and for 550 infants at age 12 months. Twenty-two mothers participated with two children (twins or siblings). Since results were not different after random exclusion of one of these siblings, they were included in the analyses. In total, at least one neurodevelopmental outcome was available in 775 children (99.6% of 778).

Determinants

We performed postnatal cranial ultrasounds with a commercially available multifrequency electronic transducer usable for three-dimensional volume acquisition (Voluson 730 Expert, GE Healthcare, Waukesha WI, USA). The probe was positioned on the anterior fontanel and a volume box was placed at the level of the foramen of Monro in a symmetrical coronal section. We scanned a pyramid shaped volume of the brain tissue and measured the volume of the lateral ventricular system offline, which was described previously in detail[16]. In short, the volume of the ventricular frontal horns, ventricular body and trigone on both sides was quantified in milliliters. Four raters manually traced the left and right ventricles. For reliability analyses, the raters each segmented 20 images twice and images of another 20 children were rated by all four raters. The intra- and inter-observer reliability of the volumetric measurements was very high, partly due to the high variance in ventricular volume within our population. Intra-observer intraclass correlation coefficients varied from 0.989 to 0.993 for the right ventricle and from 0.992 to 0.997 for the left ventricle. Inter-observer intraclass correlation coefficients were 0.950 for the right ventricle and 0.981 for the left ventricle. Coefficients of variation, defined as the ratio of the standard deviation of the measurement error and the overall mean, ranged from 7.5% for measurements in the largest ventricular volumes (highest quartile) to 26.2% for measurements in the smallest ventricular volumes (lowest quartile). In the total sample, median ventricular volume (95% range) was 0.8 (0.2 – 3.1) ml.

Neuromotor outcome

Trained research assistants, who were blinded to the ultrasound findings, performed age-adapted neurodevelopmental assessments at median (95% range) ages 6 (4 – 12) weeks, 6 (5 – 8) months, and 14 (13 – 17) months. These assessments were based on Touwen's neurodevelopmental examination in infancy [17] as adapted by De Groot et al. [18] and included, depending on the age version, 17 to 22 items of muscle tone. Most tone items were scored as normal, low or high, for example during ventral or vertical suspension, during the traction test, or when lying or sitting.

We used the scales Gross Motor and Fine Motor of the Minnesota Infant Development Inventory [19, 20] to assess the attainment of motor milestones by parental observation. Examples of items are 'rolls over from stomach to back', or 'pulls self to standing position' for gross motor development and 'picks up objects with one hand' or 'picks up small objects using thumb and finger grasp' for fine motor development. The instrument includes monthly developmental milestones. The accessory age level was defined by the age when at least 75 percent of children in an American norm sample had mastered this skill [20]. In the 6-month-questionnaire, we used 7-8 items from the Gross and Fine Motor scales, which ranged from the 3-month age level to the 9-month age level. In the 12-month-questionnaire, we used the 11-12 items ranging from the 6-month to the 18-month age level. Parents were unaware of the age levels of the motor milestones and only reported for each skill whether their child was able to perform these motor skills at completing time of the questionnaire. Since there are no European norms, we used the 75% age levels from the original instrument to determine delay or lead in motor development. This age norm level of the highest attained milestone of an individual child was subtracted from its chronological age. A negative value indicates a lead in motor development, a positive difference indicates a delay in motor milestone attainment.

Covariates

Date of birth, birth weight, gender, Apgar scores, and mode of delivery were obtained from midwife and hospital registries at birth. We established gestational age by fetal ultrasound. During the 6-week visit, we measured infant fronto-occipital head circumference (cm). Maternal age and maternal educational level were determined at enrolment.

Statistical analyses

We log-transformed ventricular volume to achieve normality and calculated standard deviation scores. Mean (and standard deviation) of log-transformed ventricular volume was –0.27 (0.74). Regarding the neuromotor assessments, we summed per individual all deviant low and high muscle tone items. We dichotomized the neuromotor examinations, since the resulting scores were right-skewed and could not be transformed to satisfy the assumption of normality. We defined a non-optimal score as the highest tertile of non-optimal item scores to detect subtle variations in motor development. However, in some instances few children had non-optimal scores, e.g. only 6% of the children had one or more non-optimal high muscle tone items at age 6 months. The association between muscle tone, as assessed by the research assistants, and parent-reported motor milestone attainment was analyzed using multilevel linear regression.

The associations between ventricular volume and neuromotor outcome were analyzed using logistic and linear regression, and multilevel models to account for the dependency between measurements at different time-points in one subject [21]. We tested the inclusion of a quadratic term for ventricular volume to account for the possibility that the associations between ventricular volume and adverse motor outcomes were curvilinear. Since the resulting regression coefficients cannot be interpreted easily, and to visualize our findings, we tested the association of quartiles of ventricular volume with neuromotor development.

All associations were controlled for head circumference at time of measurement of ventricular size, and for the other potential confounders. Statistical analyses were carried out using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA) and SAS v.8.2 (Stata Corporation, College Station, TX, USA), including the PROC MIXED and NLMIXED module for longitudinal multilevel analysis.

Results

Table 1 presents the characteristics of participating children. Of this sample, 52.1% was male. Birth weight and head circumference were larger in boys than in girls (t=2.6, p-value = 0.01 for birth weight and t=8.1, p-value < 0.001 for head circumference). In the total group, gestational duration ranged from 28.9 to 43.4 weeks. Thirty-six (4.6%) children were born before 37 weeks of gestation. In our sample, more than 90% attained the fine motor developmental milestones at age 6 and 12 months, as compared to 75% of the children in the American norm sample. The relation between low muscle tone and motor milestone attainment was moderate. Children who scored non-optimal on low muscle tone at one or more neuromotor assessment showed 0.33 (95% confidence interval (CI): 0.07 - 0.59, p-value 0.01) months delay in gross motor milestone attainment and 0.13 (95% CI: -0.02 - 0.28, p-value 0.09) months delay in fine motor milestone attainment.

Table 2 presents the association between ventricular volume at the age of 6 weeks and neuromotor development as assessed by research assistants at ages 6 weeks, 6 months, and 14 months. The association between the volume of the cerebral ventricular system and low muscle tone shows a J-shape. To visualize this relationship, we divided ventricular volume in quartiles (figure 1). Low muscle tone symptoms were more common in infants in the lowest quartile of ventricular volume (adjusted odds ratio 1.5, 95% CI 1.0 – 2.2) and in infants in the highest quartile of ventricular volume (adjusted odds ratio 2.2, 95% CI 1.5 – 3.3), compared to infants in the mid-low quartile of ventricular volume. The associations of ventricular volume with high muscle tone did not reach significance level.

Table 3 presents the association between ventricular volume in young infancy and the parent-reported attainment of motor milestones at age 6 and 12 months. Ventricular volume was linearly related to gross motor milestone attainment. Children showed 0.2 months delay

Table 1. Subject characteristics

	Boys (n=404)	Girls (n=371)
Gestational age at birth, weeks	40.1 (35.7 – 42.4)	40.1 (35.6 – 42.4)
Birth weight, grams	3,523 (539)	3,424 (550)
Apgar score 1 minute after birth	9 (6 – 10)	9 (6 – 10)
Head circumference at visit 1, cm	39.0 (1.3)	38.0 (1.4)
Maternal age, years	31.6 (4.1)	32.4 (3.8)
Maternal educational level		
Primary, %	1.2	2.2
Secondary, %	34.2	31.5
High, %	64.6	66.3
Mode of delivery		
Spontaneous vaginal, %	65.5	67.0
Instrumental vaginal, %	22.7	17.7
Caesarean section, %	11.9	15.4
Neuromotor assessments		
Low muscle tone symptoms 6 weeks, %	31.4	25.7
High muscle tone symptoms 6 weeks, %	14.6	11.5
Low muscle tone symptoms 6 months, %	6.8	6.3
High muscle tone symptoms 6 months, %	6.8	5.7
Low muscle tone symptoms 14 months, %	16.2	14.5
High muscle tone symptoms 14 months, %	6.6	4.4
Parent-reported milestones		
Attained gross motor 6-month milestone, %	76.1	77.4
Attained fine motor 6-month milestone, %	99.2	98.3
Attained gross motor 12-month milestone, %	78.1	74.4
Attained fine motor 12-month milestone, %	94.1	96.2

Values are means (SD) for continuous normally distributed variables, medians (95% range) for continuous non-normally distributed variables and percentages for categorical variables.

Table 2. Associations between ventricular volume and neuromotor development from 6 weeks to 14 months of age

	Non-optimal neuromotor development	
	Low muscle tone Odds ratio (95% CI)	High muscle tone Odds ratio (95% CI)
Linear model:		
Ventricular volume, per SD	1.2 (1.0 – 1.4)*	0.8 (0.8 – 1.2)
Quadratic model:		
Ventricular volume, per SD	1.2 (1.1 – 1.4)**	-
Ventricular volume^2, per SD^2	1.1 (1.0 – 1.2)**	-

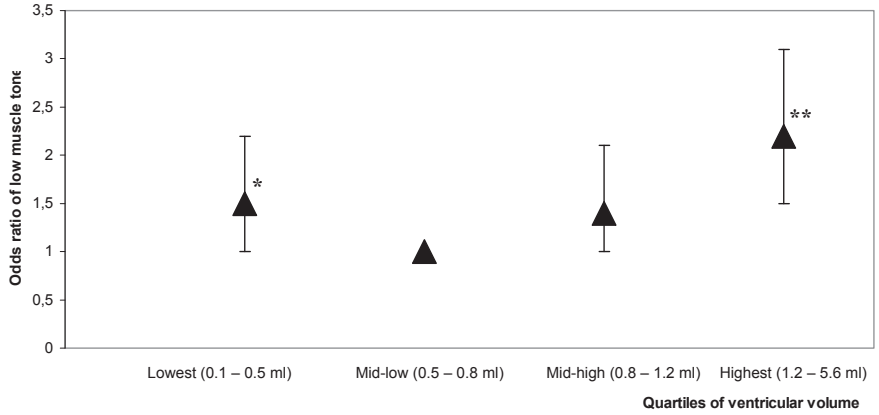
CI = Confidence interval

Values are odds ratios from repeated measurements logistic regression analyses, adjusted for head circumference when cranial ultrasounds were performed, gender, age at time of neurodevelopmental assessment, gestational age at birth, birth weight, maternal age, maternal educational level, Apgar score 1 minute after birth and mode of delivery. Ventricular volume was log-transformed to normalize the distribution, from which z-scores were calculated. The optimum in the quadratic model of low muscle tone was –0.89.

* p-value < 0.05

** p-value < 0.01.

Figure 1. The association between quartiles of infant ventricular volume and the risk of low muscle tone



Odds ratios and 95% confidence intervals are derived from multilevel logistic regression analysis adjusted for head circumference at time of cranial ultrasound, gender, age, gestational age at birth, birth weight, maternal age, maternal educational level, Apgar score 1 minute after birth and mode of delivery. Each quartile included 194 children.

* p-value < 0.05, ** p-value < 0.01.

In the model with continuous ventricular volume, the p-value of the quadratic term was 0.003.

in gross motor milestone attainment per standard deviation ventricular volume. The association between ventricular volume and fine motor milestone attainment was U-shaped (table 3). Figure 2 illustrates the association between ventricular volume and attainment of an

Table 3. Associations between ventricular volume and parental observations of motor milestones

	Motor development delay (in months)					
	Gross motor milestone attainment			Fine motor milestone attainment		
	6 months β (95% CI)	12 months β (95% CI)	Both ages combined β (95% CI)	6 months β (95% CI)	12 months β (95% CI)	Both ages combined β (95% CI)
Linear model:						
Ventricular volume, per SD	0.2 (-0.0; 0.4)	0.3 (0.1; 0.5)**	0.2 (0.1; 0.4)**	0.1 (-0.0; 0.2)	-0.1 (-0.3; 0.1)	0.0 (-0.1; 0.2)
Quadratic model:						
Ventricular volume, per SD	-	-	-	0.1 (0.0; 0.2)*	-0.0 (-0.2; 0.1)	0.1 (-0.0; 0.2)
Ventricular volume^2, per SD^2	-	-	-	0.1 (0.0; 0.2)*	0.1 (0.0; 0.2)*	0.1 (0.0; 0.2)**

CI = Confidence interval

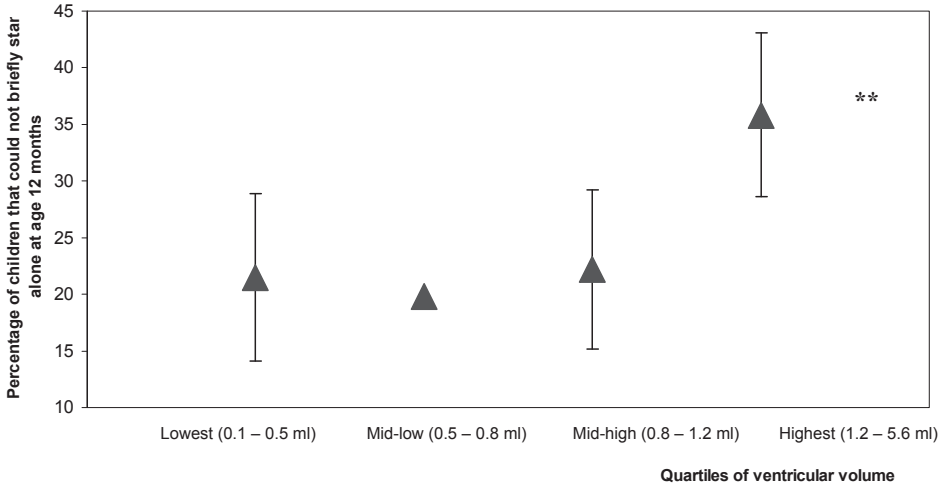
Values are regression coefficients from repeated measurements multivariable linear regression and reflect the delay in months of gross or fine motor milestone attainment per standard deviation of postnatal ventricular volume. Models were adjusted for head circumference when cranial ultrasounds were performed, gender, age at time of completing questionnaire, gestational age at birth, birth weight, maternal age, maternal educational level, Apgar score 1 minute after birth and mode of delivery.

Ventricular volume was log-transformed to normalize the distribution, from which z-scores were derived. The optimum in the quadratic multilevel model for fine motor milestone attainment was –0.34.

* p-value < 0.05.

** p-value < 0.01.

Figure 2. The association between quartiles of ventricular volume and attainment of the 12-month gross motor milestone 'stands alone, briefly'



Percentages and 95% confidence intervals are derived from analysis of covariance and adjusted for head circumference at time of cranial ultrasound, age, gender, gestational age at birth, birth weight, maternal age, maternal educational level, Apgar score 1 minute after birth and mode of delivery. Numbers per quartile: 133 in lowest and mid-low quartile, 146 children in mid-high quartile, and 141 children in the highest quartile.
* p-value < 0.05, ** p-value < 0.01.

important gross motor milestone 'stands alone, briefly'. At an average age of 12 months, 36% of the children in the highest quartile of ventricular volume were not able to stand without support. In the other three quartiles of ventricular volume, 20 – 22% of 12-month-old children had not reached this milestone.

When we excluded premature born children (n=36) from the analyses, results did not change (data not shown).

Discussion

This study shows that lateral ventricular volume in young infancy predicted neurodevelopment, whether measured by trained research assistants or reported by parents at different time-points until 14 months of age. The associations of ventricular volume with low muscle tone and fine motor milestone attainment were curvilinear (J- or U-shape), indicating that both larger and smaller ventricular volume was related to non-optimal motor development. In contrast, only larger ventricular volume was related to delays in gross motor milestone attainment.

The presence of small abnormalities in neuromotor development is thought to be a marker of an increased risk for cognitive deficits and psychiatric problems [22]. Studies in clinical samples of premature born children and studies in community-dwelling subjects

have demonstrated that neurological examination, motor tests, and reported achievement of developmental milestones can identify children at risk of learning and behavioral problems [4, 7-9, 22-24]. In premature born children, studies revealed that most neurodevelopmental disabilities have neuroanatomical substrates [11, 25, 26], but the association between brain anatomy and motor function has hardly been studied in the normal population. However, our findings that infants with larger ventricular volumes have lower muscle tone, and larger delay in gross and fine motor development are generally in line with studies in preterms. Inder et al. showed that cerebrospinal fluid volumes were increased in preterm infants with moderate-severe disability [12]. Dyet et al. found lower developmental quotients in extremely preterm infants with ventricular dilatation after intraventricular hemorrhage compared to infants without ventricular dilatation [26]. Peterson et al. described strong correlations of neurodevelopment at age 18 months with white matter volumes in the sensorimotor and midtemporal regions, but not with ventricular volumes [11].

In preterm infants with brain injury, increased cerebrospinal fluid volume signals decreased cerebral volume [11, 27]. However, a recent study in preterms without brain injury and with appropriate-for-gestational-age head circumference, showed that increased cerebrospinal fluid volume can also be accompanied by normal cerebral volume [13]. Larger cerebrospinal fluid volume, adjusted for head circumference, may therefore be a sign of immaturity with decreased growth of gray and white matter, which is reflected in hypotonicity and delayed achievement of motor milestones.

Our J- and U-shaped findings indicate that the other end of the distribution, i.e. small ventricular volume even if adjusted for head circumference, can also be a sign of immaturity of the brain. Since this association has not been described before, we can only speculate about the underlying mechanism. A possible explanation is reduced elimination of neurons and synapses, which are known to be produced in excess during prenatal development of the nervous system [28, 29].

Our findings need to be interpreted cautiously with regard to clinical impairment. The effect sizes of ventricular volumes to delays in achievement of gross and fine motor milestones were only small. Furthermore, lower muscle tone was defined as being in the upper tertile of non-optimal item scores, which usually signifies only 2 or more non-optimal items. This upper tertile represents minor variations and cannot be used to define developmental disability. Moreover, due to the low prevalence of non-optimal items on hypertonicity, although expected in a normal population, our study probably lacks power to demonstrate effects of cerebral morphology on high muscle tone.

The strengths of this study are the large number of subjects, the repeatedly measured neuromotor outcomes, and the use of both standardized examinations and parental reports. Although the mutual association between low muscle tone as examined by research assistants and parent-reported milestone attainment was relatively small, our findings of linear and curvilinear associations between lateral ventricles and neuromotor development were

consistent over different observers. Finally, excluding premature born children from our analysis revealed similar results, which shows that the high risk of brain lesions in preterms did not explain our findings. However, some methodological limitations need to be discussed. Subjects with more severe neurodevelopmental delays or complicated births were less likely to visit the research center than healthy infants. Caution is therefore required in generalizing our findings to clinical samples. Second, the lower resolution of ultrasound compared to magnetic resonance imaging necessitates manual tracing of ventricles. However, we showed a rather high reproducibility of the ventricular measurements. Third, the research assistants who assessed muscle tone, though well trained by an experienced child physiotherapist, were not experienced in assessing neurological examinations in clinical settings. Despite this uncertainty in the neurodevelopmental assessments, however, the consistency in findings across different time-points and compared to parent-reported motor milestone attainment is striking.

In conclusion, our findings provide direct epidemiological evidence that early non-optimal motor development has neurostructural correlates in normal infants. Not only large ventricular volume, but also small volume of the cerebral ventricular system, may signal immaturity of the brain and is a risk indicator for later neurodevelopmental problems. While it may be premature to speculate about clinical implications such as cranial ultrasound screening, our results suggest that it is worthwhile to follow-up chance findings of large ventricular volume in full-term infants. Ideally, future studies should extend magnetic resonance imaging techniques to samples of term and preterm infants to identify underlying mechanisms. Furthermore, the long-term consequences of small alterations in brain volumes for other developmental domains, such as cognitive and behavioral development, need to be studied.

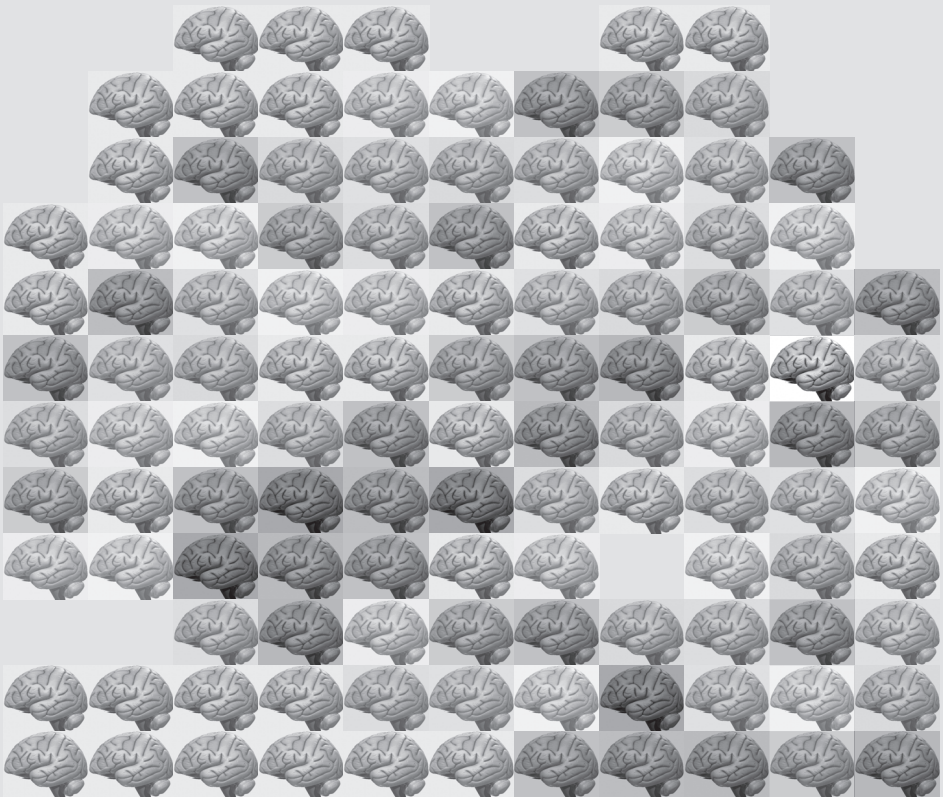
References

1. Aylward, G.P., *Neurodevelopmental outcomes of infants born prematurely.* J Dev Behav Pediatr, 2005. 26(6): p. 427-40.
2. Pedersen, S.J., K. Sommerfelt, and T. Markestad, *Early motor development of premature infants with birthweight less than 2000 grams.* Acta Paediatr, 2000. 89(12): p. 1456-61.
3. Brandt, I., et al., *Transient abnormal neurologic signs (TANS) in a longitudinal study of very low birth weight preterm infants.* Early Hum Dev, 2000. 59(2): p. 107-26.
4. Georgieff, M.K., et al., *Abnormal truncal muscle tone as a useful early marker for developmental delay in low birth weight infants.* Pediatrics, 1986. 77(5): p. 659-63.
5. Samsom, J.F., et al., *Muscle power development during the first year of life predicts neuromotor behaviour at 7 years in preterm born high-risk infants.* Early Hum Dev, 2002. 68(2): p. 103-18.
6. Groen, S.E., et al., *General movements in early infancy predict neuromotor development at 9 to 12 years of age.* Dev Med Child Neurol, 2005. 47(11): p. 731-8.
7. Hadders-Algra, M. and A.M. Groothuis, *Quality of general movements in infancy is related to neurological dysfunction, ADHD, and aggressive behaviour.* Dev Med Child Neurol, 1999. 41(6): p. 381-91.
8. Murray, G.K., et al., *Infant motor development is associated with adult cognitive categorisation in a longitudinal birth cohort study.* J Child Psychol Psychiatry, 2006. 47(1): p. 25-9.
9. Murray, G.K., et al., *Infant developmental milestones and subsequent cognitive function.* Ann Neurol, 2007.
10. Cannon, M., et al., *Evidence for early-childhood, pan-developmental impairment specific to schizophreniform disorder: results from a longitudinal birth cohort.* Arch Gen Psychiatry, 2002. 59(5): p. 449-56.
11. Peterson, B.S., et al., *Regional brain volumes and their later neurodevelopmental correlates in term and preterm infants.* Pediatrics, 2003. 111(5 Pt 1): p. 939-48.
12. Inder, T.E., et al., *Abnormal cerebral structure is present at term in premature infants.* Pediatrics, 2005. 115(2): p. 286-94.
13. Mewes, A.U., et al., *Regional brain development in serial magnetic resonance imaging of low-risk preterm infants.* Pediatrics, 2006. 118(1): p. 23-33.
14. Gilmore, J.H., et al., *Mild ventriculomegaly detected in utero with ultrasound: clinical associations and implications for schizophrenia.* Schizophr Res, 1998. 33(3): p. 133-40.
15. Jaddoe, V.W., et al., *The Generation R Study: Design and cohort profile.* Eur J Epidemiol, 2006. 21(6): p. 475-84.
16. Roza, S.J., et al., *Foetal growth determines cerebral ventricular volume in infants The Generation R Study.* Neuroimage, 2007.
17. Touwen, B.C., *Neurological development in infancy*. In: Clinics in developmental medicine. 1976, London: Heinemann.
18. de Groot, L., B. Hopkins, and B.C. Touwen, *A method to assess the development of muscle power in preterms after term age.* Neuropediatrics, 1992. 23(4): p. 172-9.
19. Ireton, H. and E. Thwing, *The Minnesota Infant Development Inventory*. 1980, Minneapolis, MN: Behavior Science Systems.
20. Ireton, H.R., *Child Development Review and the Child Development Inventories*, in *Child Development Inventories in Education and Health Care: Screening and Assessing Young Children*, H.R. Ireton, Editor. 1997, Behavior Science Systems: Minneapolis, MN. p. 3-15.
21. Twisk, J.W.R., *Applied Multilevel Analysis. A practical Guide*. 2006, Cambridge, UK: University Press.
22. Breslau, N., et al., *Neurologic soft signs and low birthweight: their association and neuropsychiatric implications.* Biol Psychiatry, 2000. 47(1): p. 71-9.

23. Lindahl, E., K. Michelsson, and M. Donner, *Prediction of early school-age problems by a preschool neurodevelopmental examination of children at risk neonatally.* Dev Med Child Neurol, 1988. 30(6): p. 723-34.
24. Pine, D.S., et al., *Neurological soft signs: one-year stability and relationship to psychiatric symptoms in boys.* J Am Acad Child Adolesc Psychiatry, 1997. 36(11): p. 1579-86.
25. Woodward, L.J., et al., *Neonatal MRI to predict neurodevelopmental outcomes in preterm infants.* N Engl J Med, 2006. 355(7): p. 685-94.
26. Dyet, L.E., et al., *Natural history of brain lesions in extremely preterm infants studied with serial magnetic resonance imaging from birth and neurodevelopmental assessment.* Pediatrics, 2006. 118(2): p. 536-48.
27. Inder, T.E., et al., *Periventricular white matter injury in the premature infant is followed by reduced cerebral cortical gray matter volume at term.* Ann Neurol, 1999. 46(5): p. 755-60.
28. Chugani, H.T., *Biological basis of emotions: brain systems and brain development.* Pediatrics, 1998. 102(5 Suppl E): p. 1225-9.
29. Webb, S.J., C.S. Monk, and C.A. Nelson, *Mechanisms of postnatal neurobiological development: implications for human development.* Dev Neuropsychol, 2001. 19(2): p. 147-71.

Chapter 5

General discussion



The main aims of this thesis were: 1) to explore the determinants of brain development in fetal and early postnatal life, 2) to assess whether subtle brain abnormalities in fetuses or young infants increase the risk of problem behavior and neurodevelopmental delays in young children, and 3) to study whether an adverse intrauterine environment is associated with fetal head growth or behavioral problems.

All studies in this thesis were conducted within the framework of the Generation R Study, a population-based cohort study among pregnant women and their children in Rotterdam, the Netherlands. In the present chapter, I will address methodological aspects, discuss the main findings in a broader context, and conclude with implications for clinical practice and future research.

Methodological considerations

The strengths and limitations of the separate studies in this thesis have been described in the specific chapters. Here, I will discuss more general methodological considerations that pertain to observational studies like the present.

Study design

The Generation R Study is a data collection project and falls into several cohorts, in which subjects are classified according to their exposure status and followed over time to ascertain disease incidence. The design is prospective, which gives the researcher full control of data collection and provides the opportunity to assess temporal relationships. Some studies described in this thesis can be considered as cross-sectional rather than longitudinal, given the short time-interval between measurement of the determinant and the outcome as well as the lack of a true baseline (e.g. in chapter 4.2). The Generation R Study aimed to follow-up subjects from the general population, instead of a special-exposure group, such as, for example, preterm born children. Population-based studies typically focus on exposures that a substantial proportion of the general population have experienced [1]. In the present thesis, this applies to the studies examining the effect of maternal smoking or folic acid supplement use during pregnancy. Special-exposure cohorts, on the other hand, select subjects for a study that have the same value, or nearly the same value, for a variable that might be a confounder. Earlier studies that examined the associations of signs of brain-sparing or subtle brain abnormalities with neurodevelopmental outcome used clinical samples of prematurely born children or children who were small-for-gestational-age [2-12]. These studies share the advantage of restriction, which limits confounding and enhances the ability to make a scientific inference. However, it is impossible to say whether the results of these studies apply to full-term children of normal birth weight. The epidemiological studies described in this

thesis aimed to provide support for hypotheses that are not tied to a specific population. Generalization of the association between structural brain development and behavior in a non-clinical population is based on statistical and biologic representativeness of the study sample.

However, the best route to a correct scientific inference is a valid study. Types of bias that affect validity include selection bias, information bias, and confounding bias [13]. All studies described in this thesis may be prone to these three types of bias.

Selection

Selection bias occurs when the relation between determinant and outcome is different in those who participate and those who were eligible for the study. These eligible subjects include those who do not participate. The initial response of the Generation R Study was estimated at 61% [14]. Non-response due to non-participation was not random. For example, the percentages of mothers from ethnic minorities and lower socio-economic status are smaller among the participants than expected from the population figures in Rotterdam [15]. Although one might expect that this selective non-response led to a more affluent and healthy study population, I argue that this is not necessarily the case. Over the years of data collection, the baseline response rates increased from approximately 51% in 2002 and 2003 to 68% in 2004 and 2005. During these years, the percentage of women that ever smoked decreased from 43% in 2002 and 2003 to 41% in 2004 and 38% in 2005. Of the women that had their delivery dates in 2002 or 2003, 25% smoked during the first trimester of pregnancy, whereas among the women with a delivery date in 2004 and 2005, 23% smoked during the first trimester. In pregnant women with Dutch nationality, the mean score on the Global Severity Index, a measure of psychological distress, decreased from 0.21 in 2002 to 0.18 in 2005 (unpublished data). Different reasons for (non-) participation, like health-consciousness or worries about specific disease risk, may be present in different groups of pregnant women.

In the Generation R Focus study, the response rates were even lower. The initial response of Dutch women in the Generation R Study is estimated at 68%, which is higher than the overall participation rate of 61%. However, of the eligible women for the Focus cohort, who were the Dutch women participating in the Generation R study and had their delivery date between February 2003 and August 2005, about 80% gave consent for participation in the prenatal detailed ultrasound examination [14]. This examination included the Doppler measurements, which were the main determinants in chapter 3.2. Of the 1,244 live born children, only 904 (73%) participated in the postnatal assessment at the age of 6 weeks. Of these, 778 (86%) children had an interpretable cranial ultrasound. All together, this indicates an initial response in the studies described in chapter 4 of (86% of 73% of 80% of 68%) approximately 34% of the eligible Dutch children in Rotterdam. In conclusion, the participants of the studies in chapter 4 are not a representative sample of the general population. Rather, children who underwent

a cranial ultrasound at the age of 6 weeks represent predominantly healthy, term children. The prevalence and effects of maternal smoking provide an example of possible selection effects in the Generation R Focus study during pregnancy. In the total cohort, we described a strong association between maternal smoking during pregnancy and behavioral problems at the age of 18 months. This association was largely confounded by parental socioeconomic status, national origin, and parental psychopathology (chapter 2.3). In the Focus cohort, maternal smoking in pregnancy had no effect on behavioral problems and was therefore not a confounding factor in the association between signs of fetal circulatory redistribution and behavioral problems at the age of 18 months (chapter 3.2). This is probably due to the absence of the epiphenomena of maternal smoking during pregnancy in the Focus cohort. The Focus cohort might represent a sample at low vulnerability for emotional and behavioral problems, due to the favorable environmental characteristics, which underscores the possibility of an adverse effect of maternal smoking on behavioral development in high-risk groups (as discussed in chapter 2.3). Thus, selection within the Focus study also yields advantages. In a more homogeneous group, effects of confounding are smaller and conclusions on scientific inference are easier to draw.

Apart from selective non-response, almost all studies described in this thesis suffer from selective loss to follow-up. Analysis of missing data showed consistent attrition of lower educated, younger mothers with higher psychopathology scores, and of children with lower birth weights and shorter gestation. Selective attrition only leads to bias when associations differ between those who participate and those who were lost to follow-up. Children with missing information on behavioral outcome were, according to their background risk factors, at higher vulnerability for emotional and behavioral problems. Furthermore, they differed from participating children in exposure status. Their mothers more often continued smoking during pregnancy and less often used folic acid supplements in early pregnancy. When we assume that the association between exposure to cigarette smoke and behavioral problems is larger among non-participants, our effect sizes are an underestimation of the effect in the general population. However, since the association between exposure and disease in non-participants is generally unknown, the effect of selective attrition can only be inferred.

Information

Information on the determinants and outcomes in the studies described in this thesis was mainly obtained by ultrasound examinations and parental questionnaires. Less predictable information bias occurs when misclassification of the outcome is related to the determinant, or vice versa [1]. In most of our studies, exposure data were collected before assessment of the outcome, which makes differential misclassification of the exposure unlikely. In many of the earlier chapters, the determinant was assessed using ultrasound examination, whereas mothers reported on the outcome. In general, mothers were blinded to the exposure status,

which makes differential misclassification of the outcome also less likely. Moreover, in the analyses on the association between cerebral ventricular volume and motor development (chapter 4.3), we showed consistency of results over different observers. Whether parents reported on motor milestone attainment or research assistants examined the infants, children with smaller ventricles and children with larger ventricles had a higher risk of neurodevelopmental delay. However, in chapter 2.3 and 2.4, both information on the exposure (smoking and folic acid use) and information on behavioral outcome were obtained using mother-reports. It is unlikely that maternal report of her child's behavior is directly related to her smoking status or folic acid supplement use during pregnancy, although misclassification due to epiphenomena of smoking and folic acid supplement use might occur.

Studies using several observers of behavioral outcome are needed to clarify the issue. Agreement between different types of informants seeing children under different conditions is modest, due to variations in children's functioning across situation, and variations in adult's judgments of children's behavior [16]. However, each source contributes to the validity of the information [17]. Both in clinical settings and in epidemiological research, accurate assessment of the child's functioning must take account of variations in their behavior across situations and interaction partners. Children themselves are the most knowledgeable source to report on their own behavior and emotions, although their cognitive abilities may limit the accuracy of their reports. New methods to assess psychopathology in pre-schoolers have shown, however, that young children can validly report on some key aspects of both externalizing and internalizing behavior [18]. Parents and teachers may be able to report more objectively on the behavior of children, but they may be unaware of actions that occur outside of the home-setting or classroom and of behavior and emotions that the child successfully covers [19]. Behavioral observation by clinicians or researchers is probably the most objective report of the child's behavior, but several behavioral problems are difficult to assess in a brief clinical visit [20]. In conclusion, there is no gold standard measure to assess child behavior and emotions, anyhow behavioral assessment requires multiple informants [21]. To draw definite conclusions on the effects of intrauterine environmental factors, future studies will need the integration of father-report, self-report, teacher-report, and behavioral observations.

Confounding

One of the important strengths of the Generation R Study is the measurement of many potential confounding variables. Planned studies on only one or a few topics are often forced to restrict the number of variables that can be obtained with the available personnel and time. The Generation R Study with its multidisciplinary setting and ongoing collection of data provides information on almost every variable that can be related to growth, development and health of the child and the parents.

Confounding may be considered a confusion of effects, in which the apparent effect of the exposure of interest is distorted because the effect of an extraneous factor is mistaken for or mixed with the actual exposure effect [13]. A confounding factor should be associated with both the exposure and the outcome, and cannot be an intermediate in the causal chain from exposure to outcome. When investigating the association of intrauterine environmental effects like smoking and folic acid supplement use with behavioral disorders (chapter 2.1 or 2.3), many confounders of the association were present. We showed that determinants like educational level, national origin, and parental psychopathology confounded the relation between maternal smoking and child behavior, as well as the relation between maternal folic acid supplement use and child behavior. Moreover, in chapter 3.1, we showed that maternal characteristics such as national origin, age, and height, were the most important confounding variable in the association between fetal growth and infant temperament. These examples show that control for confounding in behavioral studies often means that several social, demographic, psychological and physiological factors need to be studied. However, these examples also show the danger of overadjustment. Overadjustment can result from adjusting for an intermediate in the causal pathway between a negative intrauterine environment and infant behavior, or from adjustment for a variable that is causally related to the exposure but only correlated to the outcome. Sociodemographic and psychological factors like maternal educational level, age, psychopathology, and national origin are unlikely to be intermediates in the association between intrauterine environmental factors and child behavior. However, in the example of fetal growth and infant temperament, maternal height might be considered as a preceding factor in the causal chain from fetal growth and infant temperament. Maternal height is causally related to intrauterine growth. It may be related to infant temperament only via its effects on fetal growth. However, maternal height can also be related to maternal report of infant behavior because maternal height is an indicator of socioeconomic status and health status of the mother.

On the other hand, we may have missed potential confounders. The issue of residual confounding can never be ruled out in an epidemiological study. Particularly the study in chapter 2.4 may be subject to residual confounding, since the sum of several sociodemographic factors already led to strong attenuation of the effect of folic acid supplement use on child behavioral problems. Both the determinant and the outcome are strongly related to countless epiphenomena of socio-economic status, such as smoking, alcohol use, unfavorable housing conditions, psychopathology, unhealthy dietary intake, relational and occupational stress, etc. However, even with complete data on all these variables, it would be difficult to control for confounding without overfitting the models, since the inclusion of several factors that are highly correlated induces the problem of multicollinearity.

Problems of confounding were far less evident in the studies examining the associations of cerebral blood flow and ventricular volume with behavioral and motor development in the Focus cohort (chapter 3.2 and chapter 4). Since these biological determinants were not or

only moderately related to sociodemographic variables, the important confounding variables of our other studies did not change the measure of associations. These examples underline the benefits of studying etiological associations within a restricted and homogenous sample, like the Generation R Focus Study, which I discussed earlier.

Causality

Another issue that may pose problems in observational studies is causal inference. To determine whether an observed association is causal, temporality is arguably the most important criterion: the cause needs to precede the effect in time [22]. However, it is not always straightforward to establish whether the cause preceded the effect. In chapter 2.2, in which we described an association between maternal distress during pregnancy and infant growth, the determinant and outcome were measured in close temporal proximity. This example may represent reverse causality, i.e. the mother is anxious or depressed during pregnancy due to growth restriction of the child. Other 'causal criteria' of Hill are strength, consistency, specificity, biological gradient, plausibility, coherence, experimental evidence, and analogy [1]. Each criterion has its own problems. Strength of an association depends on the prevalence of other causal factors and on the influence of confounding variables. Smoking during pregnancy, for example, was strongly associated to behavioral problems, when the effects of important confounders are not controlled for. On the other hand, the effect sizes of structural brain variations and fetal circulatory redistribution on behavior and neuromotor development were small. Many biological causes of illness are small, but this does not necessarily represent a weak causal association. Other factors in the 'causal pie' may be more prevalent, like social disadvantage in the prediction of behavioral disorders. The small effect size reflects that, on population level, the total burden of behavioral problems is not caused by structural brain abnormalities. However, in a small proportion of cases, structural brain variations may be a causal component for neurobehavioral problems, or, on the individual level, the biological vulnerability may be necessary to the occurrence of behavioral problems.

Consistency of findings may reflect causality, but exceptions are understood best with hindsight. Although large ventricles could not be predicted by fetal growth restriction and were not related to temperamental difficulties, they were found to be related to neuromotor development. Future studies may provide information on the association between large ventricles and cognitive and behavioral problems in normal children at school age. Specificity misleadingly suggests that a relation is more likely to be causal if the exposure is related to a single outcome rather than to several outcomes. However, there is much evidence that smoking is related to many adverse health conditions.

The biologic gradient can be confounded, like in the example of smoking during pregnancy and behavioral problems. Plausibility, coherence, and analogy are vague and subjective criteria. Finally, experimental evidence is not always available. Since folic acid supplement

use is already generally considered to prevent neural tube defects, it is not ethical to start experiments like randomized controlled trials to unravel the effect of folic acid supplement use on child behavior.

In conclusion, it is not easy to determine whether an observed association is causal. The aforementioned causal criteria are only viewpoints or standards, as Hill himself already mentioned [23], and may only give positive support to inferences about causality. Although causation is an essential concept in the practice of epidemiology, yet there is no single definition on causation [24]. Necessary causes, for example, are conditions without which the effect cannot occur. Sufficient-component causes are made up of a number of components, in which none is sufficient on its own but taken together make up a cause that guarantees that the effect will occur. Perhaps the most practical definition for epidemiologists is the probabilistic one, in which causes increase the probability of its effect occurring. One should always has in mind that causal models are constructed within limits set by the researcher's definition [24].

Since biologic knowledge about epidemiologic hypotheses is often insufficient, these hypotheses are at times little more than vague statements of association between exposure and disease [22]. To cope with this vagueness, epidemiologists usually focus on testing the negation of the null hypothesis that the exposure does not have a causal relation to disease. However, any observed association may possibly rebut the null hypothesis. It is important to incorporate assumptions about hidden biological mechanisms. For example, it may be tempting to speculate that variations in ventricles represent the process that is causally responsible for neurodevelopmental delays and temperamental difficulties. However, I think it more likely that brain structure is only an indicator of the maturity of the brain, rather than a risk factor for neurodevelopmental problems.

Main findings

Intrauterine environmental influences on brain and behavior

Evidence from numerous longitudinal studies suggest that part of the vulnerability for mental disorders is shaped in fetal life [25-28]. Several intrauterine environmental factors have been studied in relation to fetal growth, brain development, and behavioral outcome. Here, we focus on three main determinants that have been well-documented to affect fetal growth and development, and include maternal smoking in pregnancy, maternal distress in pregnancy, and folic acid supplement use in the first trimester of pregnancy.

The negative effects of maternal smoking on both the pregnant woman and on the developing fetus have been well demonstrated. Maternal cigarette smoking is a risk factor for intrauterine growth restriction, perinatal morbidity and mortality, and postnatal growth

[29-31]. Animal studies showed direct effects of nicotine on the developing brain, such as altered cell proliferation and differentiation, and development of several neurotransmitter systems [32, 33]. The negative influences of prenatal cigarette smoke exposure on human brain development are mainly shown indirectly. Several researchers have studied the association between maternal smoking in pregnancy and behavioral and cognitive development in the offspring [34]. However, there is far less evidence for the direct negative effects of prenatal cigarette exposure on brain structure in human fetuses.

With regard to the effects of exposure to stress in utero, studies point to a small but reliable link between prenatal stress and smaller size at birth [35]. Experiments in animals showed fetal reduced weight gain due to exposure to stress [36]. Furthermore, studies in humans demonstrated that maternal psychological distress in pregnancy is related to an increased risk of spontaneous abortion, preterm delivery, and low birth weight [37-39]. Moreover, there is indirect evidence for the effects of prenatal maternal stress on the developing human brain since antenatal stress is associated with adverse neurobehavioral outcomes, including emotional and cognitive functioning [35].

Finally, an adequate folate status of the mother during the first trimester of pregnancy is important for the prevention of neural tube defects and for normal fetal growth [40, 41]. The neurodevelopmental consequences of folate deficiency after closure of the neural tube are less clear, although animal studies described essential roles for folates in neuronal and glial growth and proliferation, as well as in synthesis of neurotransmitters [42, 43].

We found that maternal smoking during pregnancy leads to reduced growth of the fetal head circumference and biparietal diameter, even after controlling for the effects on abdominal circumference (chapter 2.1). Furthermore, we found small structural alterations in the cerebellum and the ventricular system by prenatal cigarette exposure (chapter 2.1) However, neither growth of the cerebellum during the second half of pregnancy, nor narrowing of the ventricles, was affected by prenatal cigarette smoke exposure.

Affective symptoms and family stress also led to reduced growth of the fetal head, abdomen, and femur length (chapter 2.2). Weight at birth, a summary measure of intrauterine growth, was only affected by maternal symptoms of anxiety in mid-pregnancy (chapter 2.2).

Finally, we investigated the associations of maternal smoking and folic acid supplement use in pregnancy with child behavioral development. The observed association between maternal smoking in pregnancy and children's behavior was fully explained by national origin, parental socioeconomic status, and parental psychiatric symptoms (chapter 2.3). The confounding effect of these epiphenomena of smoking was highly consistent with regard to the dose-response relationship of maternal smoking in pregnancy. Furthermore, we showed that children of fathers who smoked in the same room as their non-smoking pregnant partner had a higher risk of behavioral problems compared to children of non-smokers. However, this effect of passive smoking was also fully explained by sociodemographic factors and parental psychopathology.

Although the association between folic acid supplement use and child behavior was also strongly confounded by maternal characteristics, children of mothers who used folic acid supplements during early pregnancy had a significantly lower risk of behavioral problems at the age of 18 months compared to children of mothers who did not use folic acid supplementation. Birth weight and fetal head growth did not mediate this association.

Our results indicate that different intrauterine environmental factors affect fetal growth, fetal brain development, and child behavioral outcome. We propose the following underlying mechanisms. First, intrauterine environmental factors may have a direct effect on the developing brain. Prenatal cigarette smoke exposure in animal experiments was associated with increased cell death, and functional alteration of nicotinic acetylcholine receptors. Although we described structural alterations in the cerebellum and the ventricular system, these changes in size were stable throughout the second half of pregnancy. This may indicate that maternal smoking leads to cell loss in these brain regions in specific vulnerability periods during gestation. Similarly, animal studies suggested that folates have direct effects on the development of the brain. Folates are involved in neurogenesis, neuronal growth and proliferation, and myelination. Finally, maternal stress may lead to disturbances in the maternal hypothalamus-pituitary-adrenal axis activity with an increased release of glucocorticoids [44]. These glucocorticoids can easily pass the placenta, and may lead to abnormalities in brain development, due to their direct neurotoxic effects or to their regulatory relationships with brain neurotransmitter systems.

A second possible mechanism is the effect of adverse intrauterine environmental factors on the uteroplacental and fetoplacental circulation, which leads to fetal hypoxia. Nicotine is known to induce vasoconstriction and decreased oxygen availability. Another component of cigarette smoke, carbon monoxide, induces higher levels of carboxyhemoglobin, which also leads to intrauterine hypoxia [29]. It has been demonstrated that cigarette smoke exposure leads to fetal growth restriction [30], which is one of the signs of chronic fetal hypoxia. We found evidence for a more general, symmetrical growth restriction including decreased head circumference due to maternal smoking during pregnancy. Maternal stress may also reduce uteroplacental blood flow because cortisol and catecholamines in particular are known to affect vessel tone [45]. Folates, finally, are also known to have vascular effects, since folic acid determines blood concentration of homocysteine. Placental vasculopathy secondary to hyperhomocysteinemia may explain the association of folate deficiency with placental abruption and preeclampsia [46].

Third, the effects of intrauterine environmental factors on neurodevelopment may be explained by gene-environment interplay, like epigenetic dysregulation of genes or gene-environment interactions. Epigenetic modifications are changes in gene expression by DNA methylation and alterations of chromatin structure [47]. Folates are known to modify the integrity and expression of genes, which, in interaction with susceptibility genes, have been proposed to be related to autism and schizophrenia [48, 49]. Similar epigenetic modifications

have been proposed to explain the long-term consequences of fetal exposure to glucocorticoids [50]. Animal studies described alterations of expression of hippocampal genes due to prenatal stress occurring during a critical period of fetal development. These genes and proteins played an important role in regulating the rate of pre-synaptic maturation and induced permanent changes in behavior of the rats [51]. Epigenetic mechanisms that explain the association between maternal smoking in pregnancy and child behavior are less well described. However, there have been several studies that proposed interacting effect of DRD4 and DAT polymorphisms with prenatal smoke exposure in the prediction of ADHD [52, 53].

Finally, the epiphenomena of lifestyle determinants in pregnancy may explain the association between intrauterine environmental factors and neurodevelopment. Several reviews described numerous confounding factors in the relation between maternal smoking and child behavior and cognition, such as parental psychopathology, co-abuse of other substances, poor prenatal care, dietary restriction, and low socio-economic status [34, 54, 55]. We showed that these factors led to complete attenuation of the effect of maternal smoking on child behavior. The effects of maternal prenatal stress and folic acid supplement use on child behavior were partly explained by sociodemographic, other dietary factors, and lifestyle-related factors. However, as I discussed earlier, it may be argued that these studies suffer from residual confounding.

Fetal adaptations to an adverse environment

Chapter 2 extended existing knowledge on the effects of specific intrauterine environmental factors on brain and behavior. Chapter 3 focused on fetal adaptations to an adverse intrauterine environment. A commonly used indicator of the intrauterine environment is birth weight. However, birth weight is a single, cross-sectional summative measure, taken at the end of a long period during which growth is rapid and exponential [56]. The same birth size can be obtained by different intrauterine growth trajectories. Birth weight is influenced by genetic factors, which account for around 40% of the variation in weight [57]. When an individual fetus is supposed to grow on the upper percentiles based on its genetic growth potential, fetal growth restriction may still lead to normal birth weight. Environmental factors affect fetal growth trajectories due to their induction of chronic fetal hypoxia [58]. The fetus responds to chronic hypoxemia with a decrease in oxygen consumption, both by decreased fetal body and breathing movements and by a fall-off in growth [59]. Fetal blood flow redistribution enhances the delivery of oxygen to vital organs. The oxygen supply of peripheral tissues is reduced ('organ sparing'), and with sustained hypoxemia, the fetus will try to maintain oxygen supply of the brain as optimal as possible ('brain sparing'). Both fetal growth restriction and circulatory signs of fetal blood flow redistribution may be considered as indicators for an adverse intrauterine environment. The studies in chapter 3 aimed to describe the associations between these indicators and behavioral outcome.

We found little indication for an association between intrauterine growth trajectories and temperamental difficulties at the age of 6 months (chapter 3.1). The negative association between birth weight and infant temperament was spurious and disappeared when national origin, maternal educational level, maternal age, and maternal height were controlled for. Full adjustment for these confounding variables diminished the effects of fetal growth trajectories on most temperamental dimensions as well. In contrast, we found a significant association between fetal circulatory redistribution and behavioral problems at the age of 18 months (chapter 3.2). Children with fetal blood flow redistribution to the frontal lobes of the brain had a higher risk of emotion regulation problems, somatic complaints, and attention problems. These findings suggest that, despite the fetal adaptive mechanism to maintain oxygen supply to brain regions that are involved in higher-order brain functions, chronic fetal hypoxia adversely affects development in this brain area.

The results from the studies described in chapter 3.1 and 3.2 seem to be contradictory. However, several important differences exist in both the design of the studies and in the biological pathway that was studied. First, the findings were from different samples. Whereas the study on intrauterine growth trajectories was conducted in the multi-ethnic total Generation R cohort, we measured Doppler indicators of fetal circulatory redistribution only in the Generation R Focus cohort. Second, we used different outcome measurements. Although several studies argued that temperamental dimensions such as activity level, negative affectivity, and early orienting play a role in the etiology and maintenance of child behavioral problems, one should be cautious in interpreting temperament as stable throughout development and predictive of behavioral and emotional problems in later childhood. However, when we studied the relation of fetal growth restriction and CBCL problem behavior, the association could be explained by the same confounding variables that attenuated the association between fetal growth restriction and temperamental difficulties (unpublished data). Moreover, when we restricted the analyses of intrauterine growth restriction and temperamental difficulties or behavioral problems to participants in the Generation R Focus study, results were very similar (unpublished data) Therefore, it is plausible that signs of fetal circulatory redistribution are better indicators of chronic fetal hypoxia than fetal growth restriction. The changes in oxygen transport may be secondary to the metabolic responses to chronic hypoxemia. Furthermore, whereas growth trajectories are strongly genetically predisposed [58], the vascular response in the cerebral and umbilical arteries is to a large extend the result of environmental factors [60].

Cerebral ventricular volume in infancy

In the last two decades, numerous studies investigated the associations between anatomical brain structure and child and adolescent psychiatric disorders [61]. Several disorders, such as ADHD, autism spectrum disorders, and schizophrenia, have been hypothesized to be neurode-

velopmental in origin [62, 63]. However, much less is known about the onset and trajectory of the subtle deviations in brain morphology in children with psychiatric disorders. Therefore, we aimed to study whether structural brain characteristics shortly after birth are related to behavioral and motor development. Cranial ultrasound imaging via the anterior fontanel is widely used by neonatologists, is noninvasive, safe, inexpensive, and can be performed at the bedside. Since we aimed to quantify brain structures, we used three-dimensional ultrasonography, which has been shown to be a reliable, accurate and valid method to quantify ventricular volume [64]. Ventricular volume was hypothesized to be a general marker of the maturity of the brain. The children in our study underwent cranial ultrasonography at the age of 6 weeks, when the anterior fontanel was still open.

We found that fetal size and growth trajectories as well as gestational age at birth partly explained the wide variability of cerebral ventricular size in normal infants. Larger head size and increased head growth during the second half of pregnancy predicted larger ventricular volume. Furthermore, the volume of the cerebral ventricular system was larger in children with longer gestation (chapter 4.1). In chapter 4.2, we describe that variations in ventricular size at the age of 6 weeks are associated with temperamental difficulties. Infants with smaller ventricular volume showed higher activity, more anger-irritability, and poorer orienting later in life. Finally, our findings in chapter 4.3 provide evidence that ventricular volume is also related to early motor development. Both larger and smaller ventricular volume were related to low muscle tone and delays in fine motor development. Delays in gross motor milestone attainment were only predicted by larger ventricular volume (chapter 4.3).

In conclusion, these results indicate that the volume of the cerebral ventricular system may signal immaturity of the brain. Although the findings in chapter 4.1 and 4.2 showed that smaller ventricles are markers of non-optimal development, the results in chapter 4.3 moderate this conclusion and indicate that both extremes of ventricular volume point to an immature brain, which we hypothesized when data collection started. Probably, several causal mechanisms determine the size of the cerebral ventricular system. Increased ventricular volumes can be the result of an obstruction to the flow of cerebrospinal fluid. This obstruction may be due to congenital malformations or to intraventricular hemorrhage [65]. Non-obstructive hydrocephaly may be caused by immature resorption of the cerebrospinal fluid in the arachnoid granulations. Ventriculomegaly secondary to white matter damage is another common cause of increased ventricular volume in premature born children. All these causes have been extensively described in clinical samples [7]. However, the volume of the ventricular system in normal infants has hardly been studied. In healthy older children and adolescents, it was already shown that ventricular volume increases with age, also after adjustment for head growth [66]. We can only speculate why small ventricular volume at the age of 6 weeks is a sign of an immature brain as well. First, smaller ventricular volumes might be the result of reduced secretion of cerebrospinal fluid, which leads to a loss of the essential expanding pressure within the ventricular system that might determine brain morphology

[67]. Second, smaller ventricular volume may be the effect of delayed or reduced apoptosis of overproduced neurons [68]. Although changes in ventricular volume have not been described in autistic children, one of the most consistent findings in these children is enlarged brain size in infancy [69, 70]. This 'brain overgrowth' in both gray and white matter of autistic children is the result of an excess of cerebral neurons with its attendant excess of axons and activated glial cells. One of the underlying hypothesized mechanisms for the enlarged brain size in autism is a delay or failure in apoptosis in frontal and other associations cortices [70].

Clinical implications

The main outcome of the studies in this thesis was infant and toddler psychopathology. Prevalence figures of psychopathology in general population studies of both toddlers and children range from 12 to 18% [71-75]. Emotional, behavioral, and adjustment disorders are the most common, whereas neurodevelopmental disorders, such as ADHD and autism, affect about 3 - 5% of children in the normal population [75]. These behavioral disorders impose a major burden on children, parents, teachers, and on society. There is still much uncertainty about the mechanisms underlying the association between risk factors, including neurobiological and environmental factors, and the development and continuity of psychopathology in children.

Many of the findings described in this thesis provide insight in the etiology of behavioral disorders. This etiologic knowledge will help us in developing adequate interventions, including primary prevention, secondary prevention, and therapeutic intervention. Here, I will focus on direct implications of the studies described. For the development of therapeutic interventions, replication and elaboration of our findings will be needed. Several of the studied determinants have already been shown to negatively influence fetal growth and infant health. Some of the adverse physical effects, e.g. of smoking during pregnancy on growth and physical health, are probably even larger than the effects on psychological health. Therefore, our findings are merely supplementary, and underscore the need of adequate prevention and intervention strategies.

This thesis showed that maternal smoking, maternal distress, and non-use of folic acid supplements during pregnancy are risk factors for reduced fetal growth, impaired head growth, and behavioral problems in infants. Current antenatal care starts after the eighth week of pregnancy [76]. Risk analysis and health education about e.g. maternal smoking are part of the first visit to the gynecologist or midwife. Midwives and obstetricians should be aware that smoking during pregnancy, also by the father, is part of a variety of vulnerability factors for brain and behavioral development in the offspring. Parental smoking during pregnancy, in combination with psychiatric symptoms and low educational level, may serve as an indicator for specific counseling by a multidisciplinary team, including a (child)

psychiatrist. Furthermore, interventions for smoking cessation may be more effective before pregnancy than during pregnancy [77, 78]. Since folic acid supplementation should also be started before conception, we recommend improving preconception prevention and intervention. The knowledge and attitudes of parents-to be related to preconception health can be improved by increasing public awareness via schools and media, routine risk assessment through screening in the primary care setting, and offering a prepregnancy visit for couples and persons planning pregnancy as a component of maternity care [78].

Certain parents may need additional counseling, interventions, or extended follow-up by gynecologists, pediatricians of child psychiatrists. For example, women who continue smoking during pregnancy, who do not use folic acid supplements during embryogenesis, or women with mental health problems, can be followed, and, in case of psychiatric symptoms, treated during pregnancy and the postnatal period.

Extended follow-up during infancy or specific interventions may also be developed for children with signs of chronic fetal hypoxia, such as fetal circulatory redistribution or intrauterine growth restriction. These factors may be incorporated in screening protocols for children at high risk of behavioral and emotional problems that can be used in primary child health care settings. Furthermore, neonatologists and pediatricians should be aware of the possible long-term negative consequences of chronic fetal hypoxia for growth and behavior.

By now, it may be premature to recommend cranial ultrasound screening in all newborns. Further research is needed on the long-term consequences of subtle brain abnormalities in normal, term infants. However, our results suggest that it is worthwhile to follow-up chance findings of very small or very large ventricular volume in full-term infants. Awareness by the clinician, as well as measurement of behavior and motor development during the first year of life in these children, might improve early identification and intervention of behavioral disorders.

Future research

This thesis revealed several findings which may generate future studies.

First, there are several other environmental factors, such as nutrition and medication use during pregnancy, which may affect human fetal brain development and behavior. Regarding nutritional factors, prospective population-based studies are needed to study the relation of maternal intake of essential vitamins, minerals, and fatty acids with pre- and postnatal brain development and with cognitive and behavioral performance in children. In addition, clinical trials are needed to prove the efficacy of dietary changes in pregnant women. For example, a clinical trial with randomization of mothers who quit use of folic acid supplements at the end of the first trimester and mothers who continue using folic acid supplements throughout

pregnancy, may provide insight in the sensitive periods of brain development when the fetus is particularly vulnerable to the effect of folate deficiency.

Second, the nature of the associations between intrauterine environmental factors and brain development is unclear. Advanced imaging techniques, both structural and functional, are needed to elaborate the knowledge on the effects of an adverse intrauterine environment on specific brain regions. For example, it would be of interest to use structural and functional MRI in the Generation R Focus cohort to show the effects of fetal redistribution to the frontal lobes. Ideally, magnetic resonance imaging should be conducted in a large sample of young children from the general population that have been prospectively followed from fetal life onwards. Prospective studies in populations that are epidemiologically ascertained may also help to identify the longitudinal trajectories of brain abnormalities that have been found in psychiatric patients. These studies would provide an important contribution to the present field of neuroimaging, which focus on patient populations or completely healthy normally developing children and adolescents.

Third, it is not unlikely that some of the effects of an intrauterine environment are transient, whereas others may persist into behavioral and learning disabilities in childhood and adolescence. The children in the Generation R Study will be followed until young adulthood, which facilitates longitudinal studies into the long-term consequences of prenatal and early postnatal factors. Adequate assessment of behavioral and cognitive problems is one of the key challenges in this field of epidemiological research. As discussed before, next to maternal reports on behavioral problems, researchers should use information from the father, the teacher, and the child itself.

Conclusion

Most psychiatric disorders are assumed to be the result of the development of deviant behavior that is influenced by genetic factors on the one hand and the accumulation of biological, psychological, and social factors on the other hand. From conception to birth, the interactions with the biological environment are most prominent, whereas from birth to adulthood interactions with the social environment have the greatest impact on development. Our results show that prenatal environmental risk factors such as maternal smoking, maternal anxiety, and dietary intake of nutrients and vitamins, may increase the probability to illness. Their effects are both direct on fetal growth and brain development (the phenotype) and indirect, since they serve as markers for chronic negative influences in postnatal life. A negative intrauterine environment is strongly linked to social background variables, which further affect the postnatal environment. However, not all children who were exposed to negative intrauterine events develop psychopathology. Neurobiological adaptations, such as

reduced fetal growth, fetal circulatory redistribution, and structural brain variations serve as indicators for the vulnerability of a child to later environmental influences.

The studies presented in this thesis demonstrate the complexity of associations between prenatal environmental factors, biological intermediates, and child behavior. The prenatal phase is an important one, and lays the foundation of development in postnatal life. However, one cannot study the effects of intrauterine life on child and adult psychopathology without taking into account the social and psychological environment after birth. Mental health and mental disorders have their fetal origins, but the best route to scientific inference of the effects of fetal programming is to integrate and elucidate the several mechanisms involved in human behavioral development.

References

1. Rothman, K.J., *Epidemiology: an introduction*. 2002, New York: Oxford University Press, Inc.
2. Scherjon, S.A., et al., *Fetal and neonatal cerebral circulation: a pulsed Doppler study.* J Perinat Med, 1992. **20**(1): p. 79-82.
3. Scherjon, S.A., et al., *Fetal brain sparing is associated with accelerated shortening of visual evoked potential latencies during early infancy.* Am J Obstet Gynecol, 1996. **175**(6): p. 1569-75.
4. Scherjon, S.A., et al., *Neurodevelopmental outcome at three years of age after fetal 'brain-sparing'.* Early Hum Dev, 1998. **52**(1): p. 67-79.
5. Scherjon, S., et al., *The discrepancy between maturation of visual-evoked potentials and cognitive outcome at five years in very preterm infants with and without hemodynamic signs of fetal brain-sparing.* Pediatrics, 2000. **105**(2): p. 385-91.
6. Peterson, B.S., et al., *Regional brain volumes and their later neurodevelopmental correlates in term and preterm infants.* Pediatrics, 2003. **111**(5 Pt 1): p. 939-48.
7. Inder, T.E., et al., *Abnormal cerebral structure is present at term in premature infants.* Pediatrics, 2005. **115**(2): p. 286-94.
8. Miller, S.P., et al., *Early brain injury in premature newborns detected with magnetic resonance imaging is associated with adverse early neurodevelopmental outcome.* J Pediatr, 2005. **147**(5): p. 609-16.
9. Dyet, L.E., et al., *Natural history of brain lesions in extremely preterm infants studied with serial magnetic resonance imaging from birth and neurodevelopmental assessment.* Pediatrics, 2006. **118**(2): p. 536-48.
10. Mewes, A.U., et al., *Regional brain development in serial magnetic resonance imaging of low-risk preterm infants.* Pediatrics, 2006. **118**(1): p. 23-33.
11. Kok, J.H., et al., *Visual function at 11 years of age in preterm-born children with and without fetal brain sparing.* Pediatrics, 2007. **119**(6): p. e1342-50.
12. Thompson, D.K., et al., *Perinatal risk factors altering regional brain structure in the preterm infant.* Brain, 2007. **130**(Pt 3): p. 667-77.
13. Rothman, K.J. and S. Greenland, *Precision and validity in epidemiologic studies*, in *Modern Epidemiology, 2nd Edition*, K.J. Rothman and S. Greenland, Editors. 1998, Liipincott Williams & Wilkins: Philadelphia. p. 115 - 134.
14. Jaddoe, V.W., et al., *The Generation R Study: Design and cohort profile.* Eur J Epidemiol, 2006. **21**(6): p. 475-84.
15. van Lith, H., *Demografische gegevens*. 2004, Center for Research and Statistics, Rotterdam (COS).
16. Verhulst, F.C. and H.M. Koot, *Child psychiatric epidemiology: concepts, methods, and findings*. 1992, Newbury Park: Sage Publications, Inc.
17. Achenbach, T.M. and C.S. Edelbrock, *Psychopathology of childhood.* Annu Rev Psychol, 1984. **35**: p. 227-56.
18. Measelle, J.R., et al., *Can children provide coherent, stable, and valid self-reports on the big five dimensions? A longitudinal study from ages 5 to 7.* J Pers Soc Psychol, 2005. **89**(1): p. 90-106.
19. Baker, L.A., et al., *Genetic and environmental bases of childhood antisocial behavior: a multi-informant twin study.* J Abnorm Psychol, 2007. **116**(2): p. 219-35.
20. Kerr, D.C., E.S. Lunkenheimer, and S.L. Olson, *Assessment of child problem behaviors by multiple informants: a longitudinal study from preschool to school entry.* J Child Psychol Psychiatry, 2007. **48**(10): p. 967-75.
21. Kraemer, H.C., et al., *A new approach to integrating data from multiple informants in psychiatric assessment and research: mixing and matching contexts and perspectives.* Am J Psychiatry, 2003. **160**(9): p. 1566-77.
22. Rothman, K.J. and S. Greenland, *Causation and causal inference*, in *Modern Epidemiology, 2nd Edition*, K.J. Rothman and S. Greenland, Editors. 1998, Lippincott Williams & Wilkins: Philadelphia. p. 7 - 28.

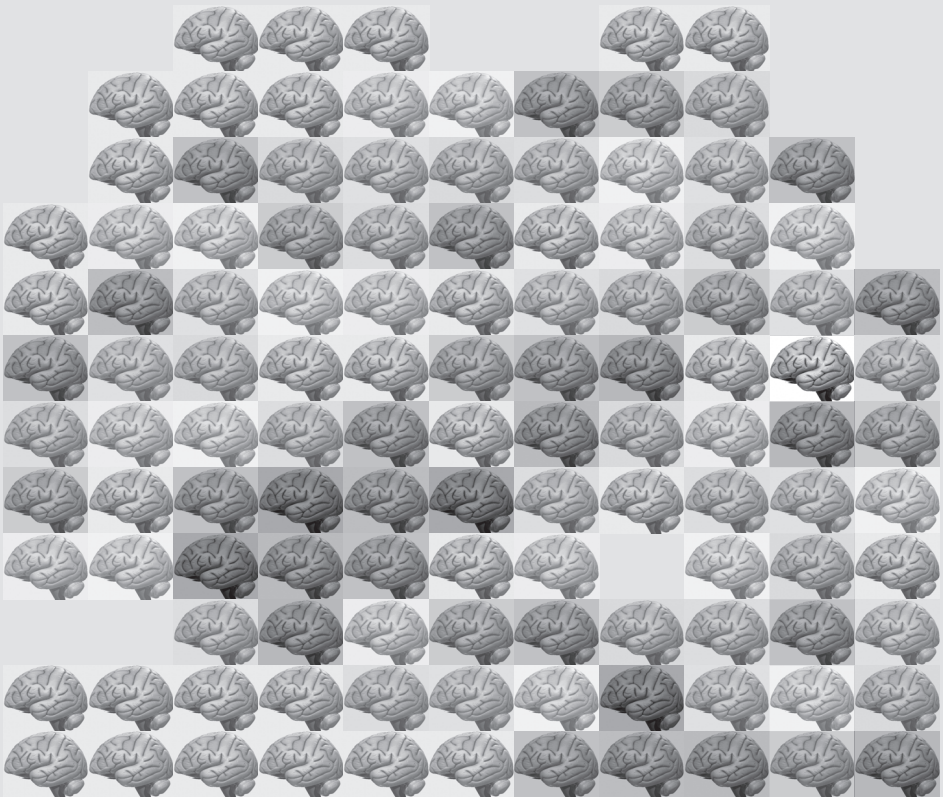
23. Hill, A.B., *The Environment and Disease: Association or Causation?* Proc R Soc Med, 1965. **58**: p. 295-300.
24. Parascandola, M. and D.L. Weed, *Causation in epidemiology.* J Epidemiol Community Health, 2001. **55**(12): p. 905-12.
25. Neugebauer, R., H.W. Hoek, and E. Susser, *Prenatal exposure to wartime famine and development of antisocial personality disorder in early adulthood.* JAMA, 1999. **282**(5): p. 455-62.
26. Susser, E., et al., *Schizophrenia after prenatal famine. Further evidence.* Arch Gen Psychiatry, 1996. **53**(1): p. 25-31.
27. Gale, C.R. and C.N. Martyn, *Birth weight and later risk of depression in a national birth cohort.* Br J Psychiatry, 2004. **184**: p. 28-33.
28. Wiles, N.J., et al., *Fetal growth and childhood behavioral problems: results from the ALSPAC cohort.* Am J Epidemiol, 2006. **163**(9): p. 829-37.
29. Abel, E.L., *Smoking during pregnancy: a review of effects on growth and development of offspring.* Hum Biol, 1980. **52**(4): p. 593-625.
30. Lieberman, E., et al., *Low birthweight at term and the timing of fetal exposure to maternal smoking.* Am J Public Health, 1994. **84**(7): p. 1127-31.
31. Jaddoe, V.W.V., et al., *Maternal smoking and fetal growth characteristics in different periods of pregnancy. The Generation R Study.* Am J Epidemiol, 2007. **in press**.
32. Slotkin, T.A., *Fetal nicotine or cocaine exposure: which one is worse?* J Pharmacol Exp Ther, 1998. **285**(3): p. 931-45.
33. Falk, L., et al., *Smoking during early pregnancy affects the expression pattern of both nicotinic and muscarinic acetylcholine receptors in human first trimester brainstem and cerebellum.* Neuroscience, 2005. **132**(2): p. 389-97.
34. Ernst, M., E.T. Moolchan, and M.L. Robinson, *Behavioral and neural consequences of prenatal exposure to nicotine.* J Am Acad Child Adolesc Psychiatry, 2001. **40**(6): p. 630-41.
35. Talge, N.M., C. Neal, and V. Glover, *Antenatal maternal stress and long-term effects on child neurodevelopment: how and why?* J Child Psychol Psychiatry, 2007. **48**(3-4): p. 245-61.
36. Lesage, J., et al., *Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behaviour disturbances in the aged rat.* J Endocrinol, 2004. **181**(2): p. 291-6.
37. Nakano, Y., et al., *Psychosocial predictors of successful delivery after unexplained recurrent spontaneous abortions: a cohort study.* Acta Psychiatr Scand, 2004. **109**(6): p. 440-6.
38. Hedegaard, M., et al., *Psychological distress in pregnancy and preterm delivery.* BMJ, 1993. **307**(6898): p. 234-9.
39. Paarlberg, K.M., et al., *Psychosocial predictors of low birthweight: a prospective study.* Br J Obstet Gynaecol, 1999. **106**(8): p. 834-41.
40. Iyengar, L. and K. Rajalakshmi, *Effect of folic acid supplement on birth weights of infants.* Am J Obstet Gynecol, 1975. **122**(3): p. 332-6.
41. Lumley, J., et al., *Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects.* Cochrane Database Syst Rev, 2001(3): p. CD001056.
42. Craciunescu, C.N., et al., *Folic acid deficiency during late gestation decreases progenitor cell proliferation and increases apoptosis in fetal mouse brain.* J Nutr, 2004. **134**(1): p. 162-6.
43. Xiao, S., et al., *Maternal folate deficiency results in selective upregulation of folate receptors and heterogeneous nuclear ribonucleoprotein-E1 associated with multiple subtle aberrations in fetal tissues.* Birth Defects Res A Clin Mol Teratol, 2005. **73**(1): p. 6-28.
44. Barbazanges, A., et al., *Maternal glucocorticoid secretion mediates long-term effects of prenatal stress.* J Neurosci, 1996. **16**(12): p. 3943-9.
45. Huizink, A.C., E.J. Mulder, and J.K. Buitelaar, *Prenatal stress and risk for psychopathology: specific effects or induction of general susceptibility?* Psychol Bull, 2004. **130**(1): p. 115-42.
46. Tamura, T. and M.F. Picciano, *Folate and human reproduction.* Am J Clin Nutr, 2006. **83**(5): p. 993-1016.

47. Muskiet, F.A. and R.F. Kemperman, *Folate and long-chain polyunsaturated fatty acids in psychiatric disease.* J Nutr Biochem, 2006. **17**(11): p. 717-27.
48. Johnson, W.G., *DNA polymorphism-diet-cofactor-development hypothesis and the gene-teratogen model for schizophrenia and other developmental disorders.* Am J Med Genet, 1999. **88**(4): p. 311-23.
49. Adams, M., et al., *Preliminary evidence for involvement of the folate gene polymorphism 19bp deletion-DHFR in occurrence of autism.* Neurosci Lett, 2007. **422**(1): p. 24-9.
50. Moritz, K.M., W.M. Boon, and E.M. Wintour, *Glucocorticoid programming of adult disease.* Cell Tissue Res, 2005. **322**(1): p. 81-8.
51. Bogoch, Y., et al., *Anxiety induced by prenatal stress is associated with suppression of hippocampal genes involved in synaptic function.* J Neurochem, 2007. **101**(4): p. 1018-30.
52. Todd, R.D. and R.J. Neuman, *Gene-Environment interactions in the development of combined type ADHD: Evidence for a synapse-based model.* Am J Med Genet B Neuropsychiatr Genet, 2007. **144**(8): p. 971-5.
53. Neuman, R.J., et al., *Prenatal smoking exposure and dopaminergic genotypes interact to cause a severe ADHD subtype.* Biol Psychiatry, 2007. **61**(12): p. 1320-8.
54. Eskenazi, B. and R. Castorina, *Association of prenatal maternal or postnatal child environmental tobacco smoke exposure and neurodevelopmental and behavioral problems in children.* Environ Health Perspect, 1999. **107**(12): p. 991-1000.
55. Linnet, K.M., et al., *Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: review of the current evidence.* Am J Psychiatry, 2003. **160**(6): p. 1028-40.
56. Bloomfield, F.H., M.H. Oliver, and J.E. Harding, *The late effects of fetal growth patterns.* Arch Dis Child Fetal Neonatal Ed, 2006. **91**(4): p. F299-304.
57. Vlietinck, R., et al., *Genetic and environmental variation in the birth weight of twins.* Behav Genet, 1989. **19**(1): p. 151-61.
58. Bryan, S.M. and P.C. Hindmarsh, *Normal and abnormal fetal growth.* Horm Res, 2006. **65 Suppl 3**: p. 19-27.
59. Richardson, B.S. and A.D. Bocking, *Metabolic and circulatory adaptations to chronic hypoxia in the fetus.* Comp Biochem Physiol A Mol Integr Physiol, 1998. **119**(3): p. 717-23.
60. Baschat, A.A., *Fetal responses to placental insufficiency: an update.* BJOG, 2004. **111**(10): p. 1031-41.
61. Giedd, J.N., et al., *Anatomic brain imaging studies of normal and abnormal brain development in children and adolescents*, in *Developmental Psychopathology (2nd ed.).: Developmental Neuroscience*, D. Cicchetti and D. Cohen, Editors. 2006, Wiley: New York. p. 127-196.
62. Filipek, P.A., *Neuroimaging in the developmental disorders: the state of the science.* J Child Psychol Psychiatry, 1999. **40**(1): p. 113-28.
63. Bradshaw, J.L. and D.M. Sheppard, *The neurodevelopmental frontostriatal disorders: evolutionary adaptiveness and anomalous lateralization.* Brain Lang, 2000. **73**(2): p. 297-320.
64. Gilmore, J.H., et al., *Infant cerebral ventricle volume: a comparison of 3-D ultrasound and magnetic resonance imaging.* Ultrasound Med Biol, 2001. **27**(8): p. 1143-6.
65. Timor-Tritsch, I.E., A. Monteagudo, and H.L. Cohen, *Ultrasonography of the prenatal and neonatal brain. 2nd edition*. 2001: McGraw-Hill Companies, Inc.
66. Lenroot, R.K. and J.N. Giedd, *Brain development in children and adolescents: insights from anatomical magnetic resonance imaging.* Neurosci Biobehav Rev, 2006. **30**(6): p. 718-29.
67. Dziegielewska, K.M., et al., *Development of the choroid plexus.* Microsc Res Tech, 2001. **52**(1): p. 5-20.
68. Webb, S.J., C.S. Monk, and C.A. Nelson, *Mechanisms of postnatal neurobiological development: implications for human development.* Dev Neuropsychol, 2001. **19**(2): p. 147-71.

69. Courchesne, E., R. Carper, and N. Akshoomoff, *Evidence of brain overgrowth in the first year of life in autism.* JAMA, 2003. **290**(3): p. 337-44.
70. Courchesne, E., et al., *Autism at the beginning: microstructural and growth abnormalities underlying the cognitive and behavioral phenotype of autism.* Dev Psychopathol, 2005. **17**(3): p. 577-97.
71. Koot, H.M. and F.C. Verhulst, *Prevalence of problem behavior in Dutch children aged 2-3.* Acta Psychiatr Scand Suppl, 1991. **367**: p. 1-37.
72. Roberts, R.E., C.C. Attkisson, and A. Rosenblatt, *Prevalence of psychopathology among children and adolescents.* Am J Psychiatry, 1998. **155**(6): p. 715-25.
73. Richman, N., J.E. Stevenson, and P.J. Graham, *Prevalence of behaviour problems in 3-year-old children: an epidemiological study in a London borough.* J Child Psychol Psychiatry, 1975. **16**(4): p. 277-87.
74. Skovgaard, A.M., et al., *The Copenhagen County child cohort: design of a longitudinal study of child mental health.* Scand J Public Health, 2005. **33**(3): p. 197-202.
75. Pallapies, D., *Trends in childhood disease.* Mutat Res, 2006. **608**(2): p. 100-11.
76. Wildschut, H.I., et al., *[Preconception care: an essential part of the care for mother and child] Preconceptiezorg: een onlosmakelijk onderdeel van de zorg voor moeder en kind.* Ned Tijdschr Geneeskd, 2006. **150**(24): p. 1326-30.
77. Women, A.C.o.H.C.f.U. and A.C.o.O. Practice, *ACOG committee opinion. Number 316, October 2005. Smoking cessation during pregnancy.* Obstet Gynecol, 2005. **106**(4): p. 883-8.
78. Johnson, K., et al., *Recommendations to improve preconception health and health care--United States. A report of the CDC/ATSDR Preconception Care Work Group and the Select Panel on Preconception Care.* MMWR Recomm Rep, 2006. **55**(RR-6): p. 1-23.

Chapter 6

Summary
Samenvatting



Several psychiatric disorders in childhood and adulthood have been hypothesized to be neurodevelopmental in origin. Numerous studies have provided evidence for subtle deviations in brain morphology in children and adults with attention-deficit hyperactivity disorder, autism spectrum disorders, and schizophrenia, compared to healthy children and adults. It is still unclear whether these subtle changes emerge in prenatal life or during brain maturation in childhood and adolescence. Findings supporting the hypothesis that (child) psychiatric disorders are related to an adverse environment in fetal life and early postnatal life include increased risk for schizophrenia in persons born after exposure to severe famine in utero and increased frequency of obstetric complications in patients with neurodevelopmental disorders.

This thesis aimed to extend existing knowledge on the prenatal and early neurodevelopmental basis of behavioral and emotional problems. The studies in this thesis were conducted in the Generation R Study, a data collection project from fetal life until young adulthood in Rotterdam, the Netherlands.

In **chapter 2**, we studied the effects of several intrauterine environmental factors on fetal growth and behavioral problems. In **chapter 2.1**, the association between maternal smoking during pregnancy and fetal head growth characteristics was investigated. When mothers continued to smoke during pregnancy, fetal head characteristics showed a growth reduction compared to fetuses of mothers who never smoked during pregnancy. The ventricles and cerebellum were also smaller in fetuses of smoking mothers, although growth per week of these structures was not affected by smoking. These results indicate that smoking during pregnancy leads to a symmetrical growth restriction, in which the development of the brain is not spared to the effects of cigarette smoke components.

In **chapter 2.2**, the effects of maternal psychological distress on fetal size and growth from mid-pregnancy until birth were studied. We found that symptoms of depression and anxiety during pregnancy led to reduced growth of the fetal head and abdomen. Moreover, family stress in mid-pregnancy was related to reduced growth of the fetal head and femur length. The results suggest that fetal growth and development in the second half of pregnancy is affected by different forms of maternal distress.

In **chapter 2.3**, we examined the hypothesis that maternal smoking during pregnancy is related to behavioral problems in young childhood. We found that children of mothers who continued smoking during pregnancy had a higher risk of behavioral problems, in particular of aggressive behavior and attention problems. However, the higher risk of behavior problems in children of smokers was fully explained by the epiphenomena of smoking, such as a low educational level, low family income and parental psychopathology. Similarly, the higher risk of behavioral problems with increasing number of cigarettes smoked by the mother attenuated after adjustment for parental characteristics. Finally, we found that children of fathers who smoked in the same room as their non-smoking pregnant partner had a higher risk of problem behavior compared to children of non-smokers. However, paternal smoking during

pregnancy is, like active maternal smoking, only a vulnerability marker and not a direct causal factor for emotional and behavioral problems.

Chapter 2.4 demonstrates that folic acid supplement use of women during early pregnancy is related to behavioral problems at the age of 18 months. Children of mothers who did not use folic acid supplements during embryogenesis had a higher risk of both internalizing and externalizing problems. Although parental characteristics accounted for a substantial proportion of this increased risk, our results suggest that folic acid supplement use protects the unborn child from behavioral and emotional problems in later life.

Chapter 3 contains two studies in which the relation between fetal adaptations to an adverse intrauterine environment and problem behavior in infancy was examined. In **chapter 3.1**, we report the association between intrauterine growth restriction and temperamental difficulties at the age of 6 months. A lower birth weight was associated with hyperactivity and prolonged duration of orienting to single objects. However, the effect of a smaller body size at birth disappeared after adjustment for maternal height, age, educational level and national origin. Similarly, the negative associations between intrauterine body weight gain and falling reactivity and activity level were spurious and disappeared after correction for maternal and child characteristics. We found little indication that intrauterine growth trajectories are related to temperamental difficulties.

Next, we studied whether fetal circulatory redistribution, also termed 'the brain-sparing effect', is associated with behavioral problems at the age of 18 months. Fetal circulatory redistribution is an adaptive response to chronic hypoxia, in case of e.g. placental insufficiency, and leads to preferential perfusion of the brain. We found in **chapter 3.2** that measures of circulatory redistribution, particularly in the anterior cerebral artery, were related to a higher risk of behavioral problems. With preferential perfusion to the frontal lobes, children had more emotional reactivity, somatic complaints, and attention problems. Our results suggest that fetal circulatory redistribution serves as a sensitive marker of chronic hypoxia and as an indicator of behavioral problems. Placental insufficiency, albeit the relative sparing of the brain to hypoxia, may have lasting consequences for neurodevelopmental outcome.

In **chapter 4**, studies into the determinants and outcomes of cerebral ventricular volume are described. We quantitatively assessed the volume of the lateral ventricular system in infants at the age of 6 weeks by using cranial ultrasonography via the anterior fontanel. **Chapter 4.1** shows that larger head size in mid- and late pregnancy, increased fetal head growth and longer gestation predicted larger ventricular volume in early infancy. These results suggest that fetal maturation partly explains the high variability in infant ventricular size. Next, we investigated the relation between ventricular volume and infant temperamental problems at the age of 6 months. The results in **chapter 4.2** show that smaller ventricular volume at the age of 6 weeks was related to higher activity, poorer orienting and more anger-irritability in 6-month-old children. Prenatal ventricular size was only marginally related to temperamental difficulties. In **chapter 4.3**, we related ventricular volume at the age of 6 weeks to neuromo-

tor development in the first 14 months of life. Both smaller and larger ventricular volume was associated with a higher risk of low muscle tone and fine motor developmental delays. Furthermore, children with larger ventricular volume more often had a delay in gross motor milestone attainment. These findings provide direct epidemiological evidence that early non-optimal motor development and infant temperamental difficulties has neurostructural correlates in normal term infants. Both small and large volume of the cerebral ventricular system may signal immaturity of the brain and serve as risk indicators for later neurodevelopmental problems.

Chapter 5 provides a general discussion of the main findings. Some of the methodological issues involved in the studies are discussed. The last part of this chapter provides some implications of our findings for the clinical setting and opportunities for further research.

Er bestaan hypothesen dat de oorsprong van verschillende psychiatrische ziekten van de kinder- en de volwassen leeftijd in de ontwikkeling van de hersenen ligt. Meerdere studies hebben bewijs geleverd voor subtiele afwijkingen in de hersenanatomie van kinderen en volwassenen met ziekten als ADHD (aandachtstekort en hyperactiviteitstoornis), autisme en autisme verwante stoornissen en schizofrenie. Het is echter nog onduidelijk of deze kleine hersenafwijkingen ontstaan in het prenatale leven of tijdens de verdere ontwikkeling van de hersenen in de kindertijd en adolescentie. Bevindingen die de hypothese ondersteunen dat (kinder)psychiatrische ziekten gerelateerd zijn aan negatieve omgevingsinvloeden tijdens de foetale en vroeg postnatale fase komen voort uit studies die een toegenomen risico op schizofrenie beschreven bij personen die geboren werden na de Hongerwinter en studies die meer bevallingscomplicaties vonden bij patiënten met neuropsychiatrische ziektebeelden.

Het doel van dit proefschrift was de bestaande kennis over de prenatale en vroege hersenontwikkelingsbasis van gedrags- en emotionele problemen uit te breiden. De studies in dit proefschrift werden uitgevoerd binnen het Generation R onderzoek, een dataverzamelingsproject vanaf de foetale fase tot in de jong-volwassenheid in Rotterdam.

In **hoofdstuk 2** bestudeerden we de effecten van verschillende intra-uteriene omgevingsinvloeden op de foetale groei en het ontstaan van gedragsproblemen. In **hoofdstuk 2.1** werd de relatie tussen roken van de moeder tijdens de zwangerschap en foetale hoofdgroei beschreven. Kinderen van moeders die tijdens de zwangerschap doorgingen met roken, hadden een afgenomen groei van het hoofd in vergelijking met kinderen van moeders die nooit rookten tijdens de zwangerschap. De hersenventrikels en het cerebellum (de kleine hersenen) waren ook kleiner in foetussen van rokende moeders, hoewel de groei van deze structuren tijdens de zwangerschap niet door roken werd beïnvloed. Deze resultaten suggereren dat roken tijdens de zwangerschap leidt tot een meer symmetrische groeivertraging, waarbij de ontwikkeling van de hersenen niet wordt gespaard voor de effecten van componenten in sigarettenrook.

In **hoofdstuk 2.2** bestudeerden we de effecten van moederlijke psychologische stress op de foetale grootte en groei vanaf het tweede trimester tot aan de geboorte. We vonden dat depressieve symptomen en angstsymptomen bij de moeder leiden tot een verminderde groei van het hoofd en de buik van de foetus. Bovendien was stress binnen het gezin rond de 20e zwangerschapsweek gerelateerd aan een verminderde hoofdgroei en groei van de bovenbenen. De resultaten suggereren dat foetale groei en ontwikkeling tijdens de tweede helft van de zwangerschap worden beïnvloed door verschillende vormen van stress bij de moeder.

In **hoofdstuk 2.3** onderzochten we de hypothese dat roken van de moeder tijdens de zwangerschap leidt tot meer gedragsproblemen op de jonge kinderleeftijd. We vonden dat kinderen van moeders die doorgingen met roken tijdens de zwangerschap een verhoogd risico hadden op gedragsproblemen, vooral op agressieve gedragingen en aandachtsproblemen. Echter, dit verhoogde risico op gedragsproblemen in kinderen van rokende

moeders kon volledig worden verklaard door diverse epifenomenen van roken, zoals een laag opleidingsniveau van de ouders, een laag gezinsinkomen en psychopathologie bij de ouders. Op dezelfde wijze verdween het verhoogde risico op gedragsproblemen bij een toename in aantal sigaretten, dat door de moeder tijdens de zwangerschap werd gerookt, wanneer werd gecorrigeerd voor deze karakteristieken van de ouders. Tenslotte vonden we dat kinderen van vaders, die binnenshuis rookten in aanwezigheid van hun zwangere vrouw, een verhoogd risico hebben op probleemgedrag in vergelijking met kinderen van niet-rokers. Roken van de vader lijkt echter, net als roken van de moeder, eerder een teken van verhoogde kwetsbaarheid dan een directe oorzaak voor het ontstaan van gedrags- en emotionele problemen te zijn.

Hoofdstuk 2.4 toont de relatie tussen foliumzuurgebruik van zwangere vrouwen tijdens het eerste trimester en gedragsproblemen bij het kind op de leeftijd van 18 maanden. Kinderen van moeders die geen foliumzuurtabletten gebruikten tijdens het eerste trimester hadden een verhoogd risico op zowel internaliserende (angst, teruggetrokken gedrag, lichamelijke klachten) als externaliserende (agressie en aandachtsproblemen) problemen. Hoewel ook hier karakteristieken van de ouders een substantieel deel van het verhoogde risico konden verklaren, suggereren onze resultaten dat foliumzuurgebruik het ongeboren kind beschermt tegen gedrags- en emotionele problemen in het latere leven.

Hoofdstuk 3 bevat twee studies naar de relatie tussen foetale aanpassingsmechanismen op een negatieve intra-uteriene omgeving en gedragsproblemen tijdens de eerste levensjaren. In **hoofdstuk 3.1** rapporteren wij het verband tussen intra-uteriene groeivertraging en een moeilijk temperament op de leeftijd van 6 maanden. Een laag geboortegewicht was geassocieerd met hyperactiviteit en een verlengde aandacht voor één enkel object. Echter, dit effect van een lager geboortegewicht verdween na correctie voor de lengte, de leeftijd, en het opleidingsniveau van de moeder en de etnische achtergrond van het kind. Op dezelfde wijze bleken de verbanden tussen intra-uteriene toename van het lichaamsgewicht enerzijds en snelheid van herstel na frustratie en activiteitsniveau anderzijds 'vals' te zijn. Deze verbanden verdwenen na correctie voor de eerder genoemde kenmerken van de moeder en het kind. We vonden dus slechts weinig bewijs dat intra-uteriene groeitrajecten gerelateerd zijn aan moeilijkheden in het temperament.

Vervolgens bestudeerden wij of foetale circulatoire redistributie, ook wel het 'hersensparend effect' genoemd, gerelateerd is aan gedragsproblemen op de leeftijd van 18 maanden. Foetale herverdeling van de bloedcirculatie is een aanpassingsreactie op chronisch zuurstoftekort, bijvoorbeeld in het geval van placentaire insufficiëntie, en leidt tot een voorkeursdoorbloeding van de hersenen. We vonden in **hoofdstuk 3.2** dat maten van bloedherverdeling, m.n. in de voorste hersenarterie, gerelateerd zijn aan een verhoogd risico op gedragsproblemen. Kinderen waarvan de frontaalkwabben tijdens de foetale fase meer doorbloed waren ten opzichte van de rest van het lichaam, hadden meer lichamelijke klachten, meer aandachtsproblemen en reageerden sterker op emotionele prikkels. Onze

resultaten suggereren dat foetale circulatoire redistributie een teken is van chronisch zuurstoftekort van de foetus. Daarmee kan de herverdeling van het bloed een indicator zijn van latere gedrags- en emotionele problemen. Placentaire insufficiëntie kan dus, ondanks het relatief hersensparend aanpassingsmechanisme op chronisch zuurstoftekort, blijvende gevolgen hebben voor de hersenontwikkeling.

In **hoofdstuk 4** worden studies naar de determinanten en uitkomsten van hersenventrikelvolume beschreven. We onderzochten het volume van het laterale hersenventrikelsysteem bij kinderen van 6 weken oud door middel van hersenechoscopie via de voorste fontanel. **Hoofdstuk 4.1** laat zien dat een groter hoofd en toegenomen hoofdgroei tijdens de tweede helft van de zwangerschap, evenals een langere zwangerschapsduur, een groter hersenventrikelvolume op de jonge kinderleeftijd voorspellen. Deze resultaten suggereren dat foetale rijping een deel van de grote variatie in ventrikelvolume op de jonge kinderleeftijd kunnen verklaren. Vervolgens onderzochten we de relatie tussen ventrikelvolume en temperamentproblemen op de leeftijd van 6 maanden. De resultaten van **hoofdstuk 4.2** laten een verband zien tussen een kleiner ventrikelvolume op de leeftijd van 6 weken en een verhoogde activiteit, een slechtere aandacht en meer prikkelbaarheid bij 6-maanden-oude kinderen. Prenatale ventrikelgrootte was slechts in beperkte mate gerelateerd aan temperamentproblemen. In **hoofdstuk 4.3** onderzochten we de relatie tussen ventrikelvolume op de leeftijd van 6 weken en de neuromotorische ontwikkeling in de eerste 14 levensmaanden. Zowel een kleiner als een groter ventrikelvolume was geassocieerd met een verhoogd risico op hypotonie (lage spierspanning) en achterstanden in de fijn motorische ontwikkeling. Bovendien hadden kinderen met een groter ventrikelvolume vaker een achterstand in het bereiken van grof motorische mijlpalen. Deze bevindingen leveren direct epidemiologisch bewijs voor het feit dat vroege motorische ontwikkelingsproblemen en temperament van baby's correleren met subtiele afwijkingen in de hersenstructuren, ook bij normale en op tijd geboren neonaten. Zowel een klein als een groot hersenventrikelvolume kan een teken zijn van onrijpheid van het brein en daarmee een aanwijzing vormen voor latere ontwikkelingsproblemen.

Hoofdstuk 5 bestaat uit een algemene discussie van de belangrijkste bevindingen. Enkele methodologische aspecten van de verschillende onderzoeken worden bediscussieerd. Het laatste deel van dit hoofdstuk beschrijft mogelijke implicaties van onze bevindingen voor de kliniek en mogelijkheden voor toekomstig onderzoek.

Dankwoord

Dit proefschrift kon slechts tot stand komen dankzij de steun van veel mensen. Allereerst mijn dank aan de tienduizenden deelnemers. Alleen door jullie deelname, het invullen van lange vragenlijsten en het veelvuldig bezoeken van onze onderzoekscentra kon een schat aan gegevens verzameld worden. Ouders, dank jullie wel voor jullie vertrouwen en vergevingsgezindheid in de eerste fase van een groot onderzoeksproject. Dit proefschrift had zonder jullie en jullie kinderen niet kunnen bestaan.

Mijn promotoren: prof. dr. F.C. Verhulst, beste Frank, dankjewel voor je bereidheid mij als jonge studente alle wetenschappelijke kansen te geven. Ik kijk met genoegen terug op de kleine gedragsgroepoverleggen, in de eerste jaren nog met gemak op jouw kamer, in de latere fase met moeite nog passend in de vergaderzaal van Generation R. Ik heb heel veel van je geleerd. Prof.dr. A. Hofman, beste Bert, bijna 10 jaar geleden kwam ik voor het eerst op jouw kamer om te solliciteren naar een plek in het MSc-programma. Ik durf te stellen dat ik een groot deel van mijn wetenschappelijke en medische carrière mede aan jou te danken heb. Het is een eer jou als promotor te hebben.

Mijn co-promotor, dr. H. Tiemeier, beste Henning, ooit begonnen we samen als 'groep' psychiatrische epidemiologie. Ik ben trots dat het je gelukt is in korte tijd zo'n grote groep promovendi, post-docs en begeleiders om je heen verzameld te hebben. Dat bevestigt eens te meer jouw wetenschappelijke visie en unieke stijl van begeleiden. Dankjewel voor je beschikbaarheid, te allen tijde, je opbouwende kritiek, je vertrouwen en alle andere levenswijsheden die je mij geleerd hebt.

Dr. P.P. Govaert en dr. M.H. Lequin dank ik voor hun klinische inzichten en beschikbaarheid, zowel tijdens de dataverzameling als tijdens de 'schrijf'fase. Paul en Maarten, ik hoop dat de 3D-echo's in een groep gezonde kinderen jullie verwachtingen hebben overtroffen.

Prof. dr. E.A.P. Steegers, beste Eric, dankjewel voor je bereidheid als secretaris van de leescommissie op te treden. I would like to thank prof.dr. R.S. Kahn and prof.dr. J.H. Gilmore for their willingness to read the manuscript and to accept the invitation to be members of the PhD-committee.

De principal investigators van de Generation R Studie, prof.dr. J.P. Mackenbach, prof. dr. E.A.P. Steegers en prof.dr. H.A. Moll, dank ik voor hun suggesties ter verbetering van de diverse artikelen.

Dr. V.W.V. Jaddoe, beste Vincent, dankjewel dat je altijd in staat bleek, hoe druk je het ook had met je vele andere activiteiten, om mijn artikelen te lezen en van commentaar te voorzien.

Alle logistiek medewerkers van Generation R dank ik hartelijk voor de enorme hoeveelheden verzet werk, zonder jullie was dataverzameling niet mogelijk geweest! Een speciaal

woord van dank aan de medewerkers van het eerste uur, ik kijk met plezier terug op de dagen, avonden en nachten dat we in de chaos orde probeerden te scheppen.

Ook de jongens (en meiden: Rachel en Claudia) van de IT en datamanagement, bedankt voor jullie inzet en tomeloze energie, waardoor Generation R elektronisch kon gaan lopen.

De echoscopisten en consulenten van de STAR maakten de dataverzameling in de prenatale fase mogelijk. In het bijzonder Eric en Bero, met plezier kijk ik terug op de middagen en avonden die we doorbrachten op de Glashaven en de Blaak.

Elianne, Laura, Saskia en Bero dank ik graag voor het maken van de vele hersenecho's en Christi, Miranda, Katja, Lies en Tonie voor de vele motorische onderzoeken. De studenten Joram, Moniek en Thamar, hebben bergen werk verzet bij het meten van de ventrikels in de gemaakte hersenecho's. Bedankt!

En dan, mijn collega-promovendi: Anne, Annemarie, Anushka, Ashna, Bero, Carmelo, Cora, Dennis, Ernst-Jan, Eszter, Fleur, Hanan, Jens, Jitske, Joost, Lamise, Lenie, Liesbeth, Lindsay, Maartje, Marianne, Mijke, Miranda, Nathalie, Nicole, Noortje, Pauline, Rianne, Sarah, Tamara en Vincent. Ik ben zo blij dat ik met jullie alle leuke en minder leuke momenten van Generation R kon delen. Noortje, 'roomie', dank voor alle reflectiemomenten! Lieve Elise, dankjewel voor je gezelligheid en warmte, ik vind het zo jammer dat we geen collega's meer zijn…

Gelukkig blijken ook psychiaters (in opleiding) gezellige borrelaars! Ab, Annemieke, Esther, Gwen, Idriss, Lonneke, Michel, Michiel, Monique, Rob, Sarah, Sieds en Toesja, dank voor jullie warme welkom! Mijke, dankjewel voor je vertrouwen. Kathelijne, Veerle, Elly, Yolanda, Joke, Marijke en het hele verpleegkundig team van P3 dank ik voor de steun in de eerste (hectische) fase van het klinisch werk. Monique, ik had mij geen fijnere collega kunnen wensen. Prof.dr. M.W. Hengeveld, beste Michiel, ik ben blij in jouw instelling tot psychiater te mogen worden opgeleid.

Kitty en Leander, dank jullie wel dat ik mij bij jullie zo thuis mag voelen! Bero en Pauline, van collega's tot vrienden tot familie, volgende kerst is er nog maar 1 GR'tje… Pauline, dankjewel dat je mij bij wilt staan tijdens mijn promotie.

Mauro, many thanks for creating this beautiful cover design out of my vague ideas. Grazie mille!! Lieve Bloeme, in vriendschap is er geen ander doel dan het verdiepen van de geest. Dankjewel dat je er bent.

Lieve pap en mam, 2008 begon niet zo rooskleurig, ik hoop dat het alsnog een mooi jaar wordt. Dank jullie wel voor jullie onaflaatbare steun en jullie liefde. Stefan, als er iemand is die mij kan opvangen, ben jij het wel. Hopelijk blijven we staan op 20 juni ☺!

Lieve Wouter, het leven is zoveel mooier met jou. Dankjewel voor alles.

About the author

Sabine Roza was born on December 16, 1979 in Rotterdam, the Netherlands. She grew up in Hellevoetsluis and passed secondary school in 1997 at the C.S.G. Jacob van Liesveldt in Hellevoetsluis. In the same year, she started to study Medicine at the 'RijksUniversitair Centrum Antwerpen' in Antwerp, Belgium. In 1998, she continued medical school at the Erasmus University Rotterdam. During medical school, she participated in an elective in Pediatrics in Guardamangia, Malta, for four weeks and she completed the Master of Science program in Clinical Epidemiology at the Netherlands Institute for Health Sciences. As part of this program, she studied for seven weeks at the Harvard School of Public Health, Boston, USA. From January 2002 until June 2002, she participated in research on the prediction of mood and anxiety disorders based on behavioral and emotional problems in childhood at the Department of Child and Adolescent Psychiatry (head: prof.dr. F.C. Verhulst). After obtaining her master degree of medicine in the summer of 2002, she started the work described in this thesis at the same department. From November 2003 until June 2005, she completed her medical training, while she continued working on her thesis. She started her specialty training in Psychiatry in January 2008, at the Department of Psychiatry of Erasmus MC, Rotterdam (head: prof.dr. M.W. Hengeveld).