THE INFLUENCE OF GENETIC, ENVIRONMENTAL AND INTRAUTERINE FACTORS ON CHILD DEVELOPMENT

The East Flanders Prospective Twin Survey (EFPTS) & The Twins and Multiple Births Association Heritability Study (TAMBAHS)

by

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ABSTRACT

I investigated the role of genetic, environmental and intrauterine factors in child development using data from two large twin studies; the East Flanders Prospective Twin Survey (EFPTS) and the Twins and Multiple Births Association Heritability Study (TAMBAHS). An association between birth weight and child development has already been established. Potential associations between other factors of the intrauterine environment and child development were investigated in this thesis.

Heritabilities of the umbilical cord, IQ, temperament and behaviour problems were estimated. Fetal characteristics, such as birth weight, placental weight and morphology, umbilical cord knots, length and insertions were investigated in relation to cognitive development in the EFPTS study. The impact of maternal pre-pregnancy weight on temperament and behaviour problems was examined in the TAMBAHS study.

High heritability estimates were observed for certain dimensions of the umbilical cord, temperament and IQ; for behaviour problems, genetic, shared and non-shared environment were important. Low birth weight and cord knotting was associated with lower IQ; an association was observed between maternal overweight and children aggressive behaviour.

The results are discussed in the context of the Developmental Origins of Health and Disease (DOHaD) hypothesis, highlighting the role of the intrauterine environment in child development.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS THESIS

Publications

1. **Antoniou EE**, Derom C, Thiery E, Fowler T, Southwood TR, Zeegers MP. The Influence of Genetic and Environmental Factors on the Etiology of the Human Umbilical cord: The East Flanders Prospective Twin Survey. **Biology of Reproduction**, 2011 Jul;85(1):137-43

2. **Antoniou EE,** Draper H, Reed K, Burls A, Southwood TR, Zeegers MP. An empirical study on the preferred size of the participant information sheet in research. **Journal of Medical Ethics**, 2011 Apr 8. Epub ahead of print

3. **Antoniou EE,** Zeegers MP. The advantage of using twin studies in epidemiological research. **British Journal of Obstetrics and Gynaecology**, 2011. In press

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1. **Antoniou EE**, Fowler T, Thiery E, Southwoord TR, van Gestel S, Jacobs N, Vlietnick R, van Os J, Rijsdijk F, Derom C, Zeegers MP. Intrauterine environment and cognitive development in young twins. **Twin Research and Human Genetics, 2010. 13 (3): p.246.** Abstract for the 13th International Congress on Twin Studies. Seoul, South Korea, June 4–7, 2010 (Invited Speaker)

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2. **Antoniou EE**, Fowler T, Reed K, Southwood TR, McCleery JP, Zeegers MP. Maternal pre-pregnancy weight and infant temperament: The Twins and Multiple Births Association Heritability Study

3. **Antoniou EE**, Fowler T, Reed K, Southwood TR, McCleery JP, Zeegers MP. Maternal pre-pregnancy weight and behaviour problems in preschool children: The Twins and Multiple Births Association Heritability Study

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TABLE OF CONTENTS

THE TWINS AND MULTIPLE BIRTHS ASSOCIATION HERITABILITY STUDY

Chapter 5: Methods: The Twins and Multiple Births Association Heritability Study

Chapter 6: An empirical study on the preferred size of the participant information sheet in research

Chapter 7: Maternal pre-pregnancy weight and infant temperament

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

95% CI	95% Confidence Internal
A	Additive
ADHD	Attention Deficit Hyperactivity Disorder
AIC	Akaike Information Criterion
BMI	Body Mass Index
C	Common
CBCL	Child Behaviour Checklist
D	Non-additive
DOHaD	Developmental Origins of Health and Disease
DZ	Dizygotic
E	Unique
EFPTS	East Flanders Prospective Twin Survey
FAQs	Frequently Asked Questions
FPI	Foot preference index
HPI	Hand preference index
IBQ-R	Infant Behaviour Questionnaire-Revised
IQ	Intelligence Quotient
L	Latent
MZ	Monozygotic
MZDC	Monozygotic dichorionic
MZMC (DA)	Monozygotic monochorionic (Diamniotic)

MZMC (MA)	Monozygotic monochorionic (Monoamniotic)
OR	Odds ratio
P	Phenotype
PIQ	Performance IQ
rDZ	Dizygotic correlation
rMZ	Monozygotic correlation
T1	Twin 1
T2	Twin 2
TAMBAHS	Twins and Multiple Births Association Heritability Stud
TIQ	Total IQ
UCMS	University Content Management System
VIQ	Verbal IQ
WHO	World Health Organization
WISC-R	Wechsler Intelligence Scale for Children-Revised

CHAPTER 1

INTRODUCTION

1.1 Summary

This thesis describes two twin studies in which the influence of genetic and environmental factors on child development for different age groups is presented. Markers of the intrauterine environment, the maternal-fetus nutritional pathway and their impact on the behavioural and cognitive development of young children were examined. A brief report on the evolvement of the importance of the intrauterine environment for later life and its implication in adult health is presented first followed by the description of the different developmental traits examined in this study and a presentation of the genetic methodology used. At the end of this chapter the aims of this thesis and an introduction to the rest of the chapters are given.

1.2 Intrauterine environment and the Developmental Origins of Health and Disease (DOHaD) approach

The intrauterine environment constitutes the first environment of the developing fetus. Previous research has focused on the adverse effects of the intrauterine environment on later life outcome and epidemiological studies on infant and adult mortality gave rise to the formation of the Developmental Origins of Health and Disease (DOHaD) hypothesis. Barker's work in this field laid the foundation with regards to the fetal origins hypothesis (1-3), often referred to as the "Barker hypothesis". His first observations began during the early 1920s while conducting an epidemiological research of distributions of disease across local authorities of England and Wales. His findings suggested that there was a positive geographic relationship between infant mortality from 1921 to 1925 and ischemic heart disease from 1968 to 1978. Several factors were implicated in the interpretation of these findings: among a few others were

the associations of the infant deaths in the 1920s with low birthweight, the dependence on adverse intrauterine environment rather than postnatal factors, the unexpected rise in heart disease with rising prosperity but the observation of lower rates in the most prosperous locations, a re-examination of the literature in maternal and infant nutrition. The first assumption of his hypothesis was formulated based on the finding that the association of infant and adults death rates must reflect variations in nutrition in early life, which are expressed pathologically on exposure to later dietary influences. It was not much later when Barker (3) investigated the link between fetal undernutrition at different stages of gestation and the variations in birth weight phenotypes and their relationship to the development of later metabolic abnormalities in adulthood. During those early exposures of the fetus to an adverse environment, fetoplacental adaptations were observed, which could only temporarily be adaptive but on the long run could lead to a permanent change of the body's structure and function and potentially increase the proneness to coronary heart problems and other diseases throughout adult life. The adaptations, which restrict the growth within the intrauterine environment as well as the occassional occurrence of mild prematurity, could be regarded as adaptive mechanisms to an adverse exposure. Thus, the association between reduced fetal growth rate, small body size at birth, and a later risk of disease may be interpreted as reflecting the long-term consequences of fetal adaptive responses (4). The main nongenetic source of intrauterine growth impairment is a reduced nutritional supply between the placenta and the fetus via the umbilical cord which could lead to low birth weight (5). Being born small however, has been associated with an increased risk of neonatal death and morbidity (6) as well as subsequent cognitive impairments (7) which is discussed below in more detail. Recent research evidence based on the fetal programming hypothesis

replicates previous findings suggesting that reduced fetal growth is associated with differences in body composition in adult life which may predispose to cardiovascular disease (8-10), type 2 diabetes mellitus (11, 12) and osteoporosis (13) in adult life. Previous findings suggest that the significance of the intrauterine environment may not be limited to physical outcomes but may also encompass other characteristics of child development and more specifically, cognitive (14, 15) and emotional/behavioural development (16). Still though, not many studies have looked at the relationship between markers of the intrauterine environment, taking into account maternal and fetal characteristics, and cognitive and emotional/behavioural development in young children.

1.3 Child development and markers of the intrauterine environment

1.3.1 Cognitive development

Cognitive functioning is considered to be an important developmental outcome. Intelligence or IQ, as most commonly cognitive functioning is referred to, is a general mental capability which involves the ability to reason, plan, solve problems, think abstractly, comprehend complex ideas, learn quickly and learn from experience. It reflects a broader and deeper capability for comprehending our surroundings, and generally "making sense" of things (17). It has been suggested that the structure and the morphology of the intrauterine environment can determine later cognitive functioning raising the debate about how much of the variance of intelligence is explained by genetic factors and how much can be explained by shared and non-shared environmental factors (18). Recently there has been an increasing interest in the identification of the implication of the intrauterine environment in the development of

psychological well-being (19, 20). Gillman (21) noted that alongside undernutrition and low birth weight as markers of prenatal aetiological pathways related to postnatal health outcomes, irregularities in the maternal-fetal supply line of nutrients could also act as a pathway which may affect later development.

This nutrient supply line is represented by the umbilical cord; some pathological and morphological abnormalities of the cord and the placenta are associated with adverse outcomes and can influence the development of cognitive abilities (18). The first environment of human beings is the one *in utero* and the developmental processes commence from this environment. The umbilical cord is formed by the fifth week of development, consisting of one vein which transfers blood rich in oxygen and nutrients from the mother to the fetus and two arteries that return deoxygenated blood and waste products, from the fetus back to the placenta. These blood vessels are enclosed and protected by a substance called the Wharton's jelly, which itself is covered by a layer membrane called the amnion (22). Although, the umbilical cord is the only organ that dies when life begins, it is one of the most important parts of the feto-placental line, having an active role in determining the manner in which the extrauterine life will begin (23). Morphological aspects of the umbilical cord, such as its length, knots, insertion to placenta, number of vessels and twisting have been associated with adverse postnatal outcomes (24-26). Based on the lack of evidence on the genetic and environmental aetiology of the umbilical cord, in chapter 4, the twin design was used to estimate the genetic and placental differences in cord morphology between the monozygotic and dizygotic twin pairs taking into account their chorionicity. Acknowledging the fact that chorionicity is a characteristic of multiple pregnancies only and that monochorionic twins compared to dichorionic are in a more disadvantaged situation, because of many

peculiarities of their intrauterine environment (i.e, twin-twin transfusion syndrome, competition for nutrients) these results cannot be generalized to singletons.

Nevertheless, there is no information on the aetiology of the umbilical cord with regards to whether genetic or environmental factors influence its development. In addition, because cord length has been found to be associated with intelligence (18), the research in this thesis was extended by investigating more aspects of the cord and the placenta, as markers of the intrauterine environment, and examining their genetic and environmental covariation with cognitive development.

As mentioned above birth weight is another intrauterine factor that has been found to be related to cognitive development. Cognitive deficits, such as learning disorders and delays in language have been associated with low birth weight (27-29). Past research has demonstrated up to 3.8 points difference in intelligence scores between high birth weight and low birth weight children (30). There is evidence moreover, that the effect of low birth weight on low intelligence holds across different birth weight ranges with the effect being however, progressively more modest (31). This suggests that genetic factors play a substantial role in explaining intelligence compared to environmental factors. In support of that, the association of birth weight with cognitive functioning in adult life was found to be similar when assessed within and between families (32). Previous findings suggest that genetic factors may contribute to the relationship between birth weight and intelligence (33) while others suggest that the environment *in utero* may be more important in explaining the relationship with cognitive development (34).

In order to further investigate this relationship I set out to examine the genetic and environmental covariation between the two traits.

In order to measure cognitive abilities, the Wechsler Intelligence Scale for Children-Revised (WISC-R) will be used in Chapter 3. Even though the use of this test has long been justified and nowadays constitute a reliable way to measure intelligence (35) in young children, there are some issues with such tests that need to be taken into account and guide the interpretation of the findings. Such issues, which relate to the testing process, include emotional tension, anxiety and unfamiliarity with the testing process, which may affect test performance. In addition, Gould described the biasing effect that the tester attitudes, qualifications and instructions can have on testing (36). Moreover, the test biases may cause cultural and racial differences. A number of biological issues, which are not necessarily measured in the test, such as malnutrition, can also result in lower psychometric intelligence (36).

However, in Chapter 3, the population studied is very homogeneous (an area in the province of East Flanders) and almost all were of Caucasian origin and in addition, it's worth mentioning that the WISC-R was validated for use in this population (37). Moreover, the test was administered separately to the two twins by trained researchers.

1.3.2 Emotional and behavioural development

Behavioural and emotional problems are present in very young children, as early as preschool (38) (39, 40) Some persistent and severe symptoms impair the child's current and future functioning and development. Because of the obvious need for early

intervention, researchers are seeking to identify potentially important markers for these serious behavioural and emotional disorders in young children. Understanding the emergence of specific symptoms in early childhood is an important step in defining targets for clinical interventions and preventative efforts (41). It is suggested that 'early emerging' behavioural traits that children show, which were later termed temperament, would be prognostic of later maladjustment (42). Emotionality, activity, and attention/orienting are three reactivity domains of temperament with relevance to Attention Deficit Hyperactivity Disorder (ADHD) and individual expression can be found in the first year of life (42). Regarding ADHD, previous findings suggest that difficulties in reactivity and regulation are present in children with the disorder both before and after the diagnosis (43). Other studies have found that negative emotionality, usually exhibited as excessive crying in the first year of life, was predictive of attentional problems and hyperactivity at school age (44, 45). From 6 months of age, anger is clearly seen as a negative emotion and difficulties with anger and its regulation are associated with ADHD from preschool through adulthood (46, 47).

Temperament plays an important role behind the aetiology of childhood anxiety disorders, and this also holds true for the models that account for the pathogenesis of depression and disruptive behaviour disorders (48, 49). Temperament is also viewed by researchers as the manifestation of children's emerging personality. Caspi and colleagues (50-52) provided empirical evidence with a longitudinal study, where children were classified into temperament groups based on the observation of their behaviour; the well-adjusted or the easy type group, which included children that were self-confident and who remained calm in novel stimuli situations, the difficult type group, which included impulsive, restless, negativist with unpredictable emotional

responses and last the group with children who were fearful and easily upset by novelty. When these temperament groups were studied again after 20 years, the results suggested that temperamental traits predicted adult personalities. In particular, children who had been classified as uncontrolled, when they were three years old were intolerant, easily upset and distrustful to other people at 26 years of age. Children described as inhibited were over-controlled and non assertive as adults. The well adjusted children still represented the normative group in adulthood.

Another longitudinal study (53) found that seven- to eight-year-old boys with high externalizing behaviour problems were also more likely to be rated high on difficultness at 6 months of age and resistance to control at 13 and 24 months of age. Caspi and colleagues showed that temperamental traits measured at age three were related to various outcomes well into adolescence and early adulthood (54, 55). In addition, Caspi and colleagues (52) found "lack of control" in 3-year-olds to be a key predictor of externalizing behaviour problems between ages nine and 15. Under-controlled preschoolers also scored high on measures of impulsiveness, danger seeking, aggression, and interpersonal alienation at age 18 (56) while inhibited preschoolers had lower scores on measures of impulsiveness, danger seeking, aggression, and social potency (52). An underlying assumption in these studies is that there is a direct link between temperament and problem behaviour later in life, both being determined by an intra-individual characteristic or trait (e.g., intra-uterine exposure, genetics) that is primarily biological in origin and expression and relatively stable. The research presented in this thesis, partly tests this assumption by investigating the relative genetic influence on temperament.

Genetic research examining the heritability of behavioural problems has mainly focused on problems within internalizing (i.e., anxiety and depression (57) (58, 59) or within externalizing arenas (i.e., aggressive and nonagressive antisocial behaviour (60). Genetic contributions to the two broadband scales (externalizing and internalizing) of the Child Behaviour Checklist (CBCL) in Dutch 3-year-olds, and also to the seven behavioural syndromes (aggressive, oppositional, overactive, withdrawn, anxious/depressed, sleep problems and somatic problems) were reported by Van der Valk and his colleagues (61, 62) and by Van den Oord (63). Van der Valk and colleagues (61) found that additive genetic factors explained 54% of the variance in externalizing behaviour and 64% of the variance in internalizing behaviour based on maternal reports of externalizing and internalizing behaviour in 3-year-old children and. Shared and non-shared environmental factors explained the remaining part of the variance. Van den Oord and his colleagues (63) suggested that additive genetic and environmental influences on the seven CBCL syndromes were present in a sample of twin pairs. Additionally, a study conducted by Derks and his colleagues (39) in a sample of 3-year-old Dutch twins showed that variation in behaviour problems in the very young shows high heritability. They found that individual differences in problem behaviour were mainly due to genetic differences, but shared and non-shared environmental factors were also present.

Research findings indicating different patterns of genetic and environmental influences on developmental difficulties, suggest further investigation of childhood emotional and behavioural difficulties, taking into consideration both aspects of genetic and environmental factors. Genetic factors have been demonstrated to be important for the

emotional and behavioural development of children, while different dimensions of environmental factors have been used in order to investigate the relationship between maternal characteristics and later development.

Recent research suggests that maternal pre-pregnancy weight has been linked with infants' high degree of distress and inattention (64-66). Nonetheless, findings from a study (67) conducted in two large cohorts show no evidence of any association between maternal weight and behavioural problems even after adjusting for environmental confounding. The inconsistent findings, along with the evidence that maternal weight as an intrauterine marker may influence later child development, renders further research important in order to identify the association between maternal weight and behaviour development and the genetic and environmental factors that influence child development using a genetically sensitive experimental design.

1.4 Twin methodology

1.4.1 Rationale for using twin studies

In order to study the contribution of genetic and environmental factors on a disease or a trait it is important to estimate how much of the disease or the trait's variance can be explained by genetic factors. According to the biometric genetic theory (68) the total variance of a quantitative trait (P) can be partitioned into the following sources of variation:

- Additive genetic influences (A), which represent the sum of the effects of the individual alleles at all loci that influence the trait.

- Non-additive genetic effects (D), which represent interactions between alleles at the same locus (dominance) or on different loci (epistasis).
- Common environmental influences (C), shared by the family members.
- Unique environmental influences (E), which result in differences between individuals (69).

Therefore, the total phenotypic variance is P = A+D+C+E

The heritability (h^2) is an index for the identification of the proportion of genetic factors accounting for the variation of a trait. Heritability is defined in a broad sense as well as in a narrow sense. Heritability in a narrow sense refers only to the additive genetic effects while heritability in a broad sense takes into account the additive as well as the non-additive (dominant) genetic effects on a trait. Most twin studies focus on the heritability of a trait in a narrow sense in order to calculate the genetic effects on a specific trait.

Often in order to estimate heritabilities, researchers use data from related individuals. Adoption and family studies are within the most common types of genetically sensitive epidemiological designs employing data drom related individuals. Family data provide a valid source for identifying familial aggregation of diseases and biological markers of genetic transmission. Nevertheless, with this design it is difficult to separate genetic from environmental influences as familial resemblance may be due to shared family environment (socio-economic status, exercise).

Adoption studies can overcome this limitation. Shared environmental effects are unlikely to have occurred if adoption occurred early in life and any resemblance between the biological affected parents and the adopted offspring indicates shared

heredity. Any resemblance between the adoptive relatives is due to shared environment. However, adoption data are not easy to obtain and the sample may be biased due to selective placement according to religious, socio-economic status and other characteristics. For these reasons a twin design is more powerfull for behavioural genetics research (69).

1.4.2 Classical twin design

The classical twin study compares the phenotypic resemblances of monozygotic (MZ) and dizygotic (DZ) twins. MZ twins inherit identical genetic material from parents, whereas DZ twins share half (like non-twins siblings) of their genetic components. MZ and DZ twins have different degrees of correlation for the genetic components A and D but the same degrees of correlation for the variance components C and E. The correlation for MZ twins is 1 for both A and D, while for DZ twins it is .5 and .25 for these components respectively. Additionally, both MZ and DZ twins correlate 1 for C while E is uncorrelated for both types of twins (69).

Higher intra-pair correlations between MZ twins (rMZ) compared to DZ twins (rDZ) indicates that genetic factors are present. However, if rDZ is greater than half of rMZ, common environmental factors are present, whereas if both rMZ and rDZ are low, then the total variance is explained by environmental factors. In the case when rDZ is less than half of rMZ, dominant genetic effects are present since D correlates perfectly for MZ but only .25 for DZ twin pairs.

Based on Falconer's formula (70), the heritability can be estimated based on the twin correlations: $h^2 = a^2 = 2(rMZ-rDZ)$, with r being the intraclass correlation coefficient. With the same way the contribution of the shared and non-shared environmental effects

can be calculated. For the shared environmental effects the formula is: c^2=rMZ-h^2 and for the non-shared environment the formula is: e^2=1 – rMZ (69).

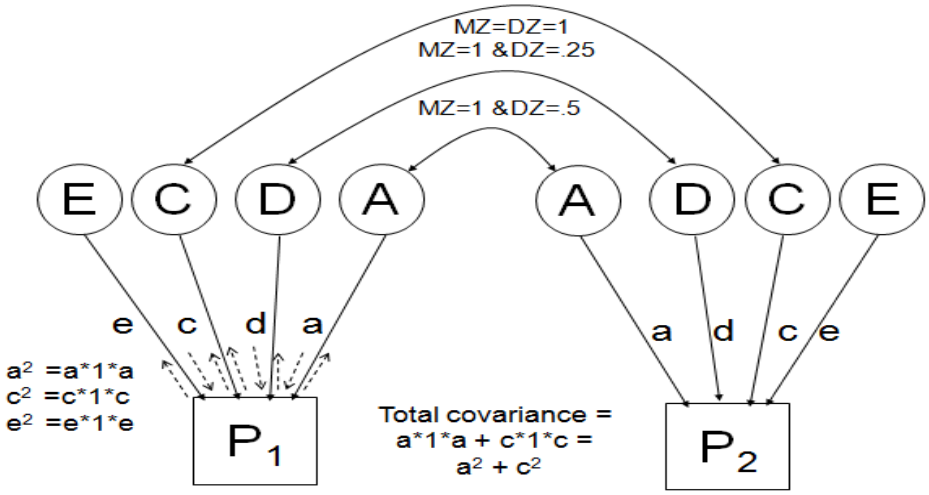

Figure 1.1 Path diagram for the basic univariate twin model (adapted from Rijdijk and Sham (69))

The observed phenotypes P_1 and P_2 for twin 1 and twin 2 respectively are shown in rectangles, while latent factors (A, D, C and E) are shown in circles. Lower case a=additive effect, d=dominant effect, c=common environmental effect and e=non-shared environmental effect are the path coefficients of observed variables on the different latent factor. For MZ the additive genetic correlation is 1 and for DZ=.5. The non-additive genetic correlation is 1 for MZ and .25 for DZ. The common environmental correlation is 1 for both MZ and DZ twins.

The above approach is however limited in dealing with the testing of further hypotheses, such as the effect of gender on variances and covariances of twins of opposite genders or giving information on how well a model fit the data and provide confiedence intervals for the estimates. Structural Equation Modelling (SEM) tests hypotheses about

relations among observed and latent variables. The use of SEM enables the estimation of model parameters by minimizing a goodness-of-fit statistic between observed and expected variances-covariances matrices. The expected variance-covariance matrix can be defined as:

ACE

		T1	T2			T1	T2
MZ	T1	$a^2 + c^2 + e^2$	$a^2 + c^2$	DZ	T1	$a^2 + c^2 + e^2$	$.5a^2 + c^2$
	T2	$a^2 + c^2$	$a^2 + c^2 + e^2$		T2	$.5a^2 + c^2$	$a^2 + c^2 + e^2$

ADE

		T1	T2			T1	T2
MZ	T1	$a^2 + d^2 + e^2$	$a^2 + d^2$	DZ	T1	$a^2 + d^2 + e^2$	$.5a^2 + .25d^2$
	T2	$a^2 + d^2$	$a^2 + d^2 + e^2$		T2	$.5a^2 + .25d^2$	$a^2 + d^2 + e^2$

Figure 1.2 Expected variance-covariance twin matrix

where a^2 represents the additive genetic variance, c^2 represents the common environmental variance, d^2 is the non-additive genetic variance and e^2 is the non-shared environmental variance. We can see above that C and D are included in the same model. However, because common environmental factors and non-additive genetic factors are confounded in MZ and DZ twins reared together, a simultaneous estimation of these two parameters is not possible. Thus, ACE and ADE models will have to be estimated separately. Modern model techniques use an iterative approach to test different models and calculate best fitting estimates. Mx is one of the programmes which can be used to fit these models. It uses mathematical functions such as chi-square or log-likelihood, which stop estimating the parameters (i.e, a^2, c^2 and e^2) once the maximum likelihood or

the minimum value of chi-square is obtained. Estimates that give values that are the least different from the observed data are considered to be the best fitting and those are accepted. These models are usually fitted to covariance matrices, but other matrices, (i.e, correlation matrices) can also be used. An extensive description of the matrix algebra and the structural equation modelling can be found in Neale and Cardon (71).

1.4.3 Heritability estimates and model fitting for categorical data

A number of traits under study however, such as medical or pathological conditions (i.e., velamentous cord insertion on the placenta, which is one of the traits of the umbilical cord studied in Chapter 4) appear to be categorical and do not conform to the simple patterns of inheritance. The liability threshold model was suggested by Falconer (72) in order to accommodate analyses with ordinal traits. A continuous liability distribution for a particular trait is assumed with this model. Just as for the continuous traits, variance decomposition can be applied to liability, in which correlations in liability are determined by path model. The heritability of the liability of the trait can then be estimated.

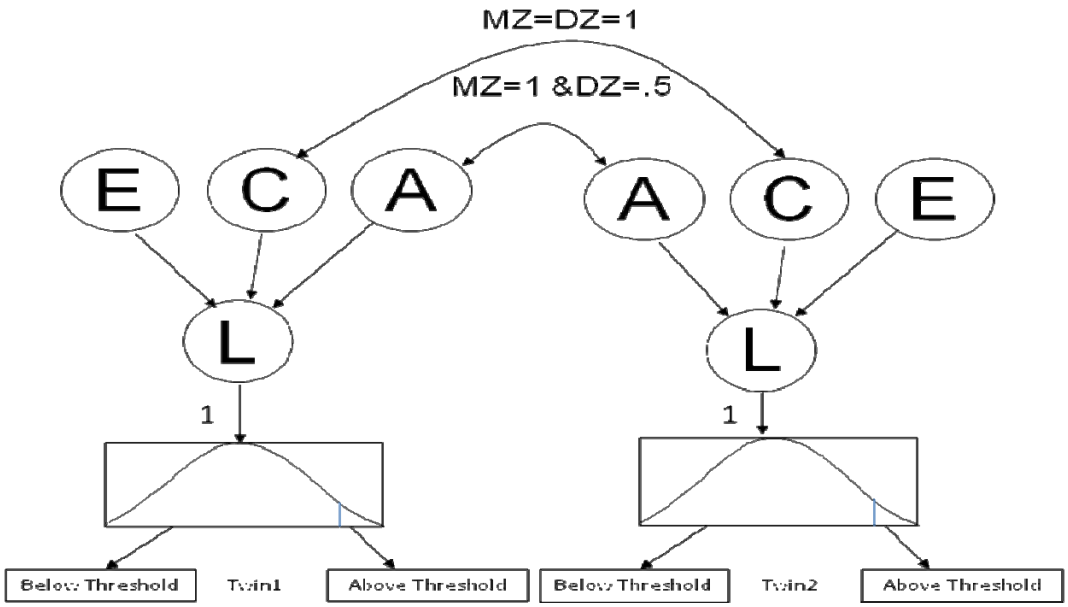

Figure 1.3 Path diagram for the univariate ordinal twin model

1.4.4 Classification of Monozygotic twins

As mentioned above, DZ twins share 50% of their genes and they arise after the fertilization of two ova. MZ twins arise from the splitting of a single fertilized ovum and thus are genetically identical. However, the moment the splitting takes place has implications for the intrauterine environment and the postnatal life (73). Four types of twins can be distinguished: 1) Monozygotic dichorionic twins (DC) arise when the splitting occurs by the 4th day post fertilization. Approximately 32% of the MZ twins are dichorionic, where each fetus has its own chorion and amnion. 2) Monozygotic monochorionic diamniotic twins (MC DA) arise when the splitting occurs between the 4th and 8th day post fertilization. Nearly 66% of the MZ twins are monochorionic-diamniotic. These twins share a common chorion but have their own amnion. 3) Monozygotic monochorionic monoamniotic (MC MA) arise when splitting takes place after the 8th day post fertilization. Around 2-3% of the MZ twins are MC MA, where

the fetuses share one chorion and one amnion. 4) When the splitting takes place after the 12th day of fertilization, conjoined twinning can occur. The occurrence of conjoined twins is very rare and it is estimated to range from 1:50.000 to 1:200.000 births (74). The type of the chorion and its influence on postnatal development has been previously studied. MC twins compared to MZ DC and DZ twins have higher perinatal mortality rate (75). Additionally, MZ MC twins have significantly lower birth weight, MZ DC somewhat lower birth weight, while DZ twins have the highest birth weight (76, 77). Unequal sharing of the placenta, vascular anastomoses and competition for nutrients may be some of the reasons why MZ MC twins experience a more adverse perinatal einvironment compared to MZ DC and DZ twins (78, 79).

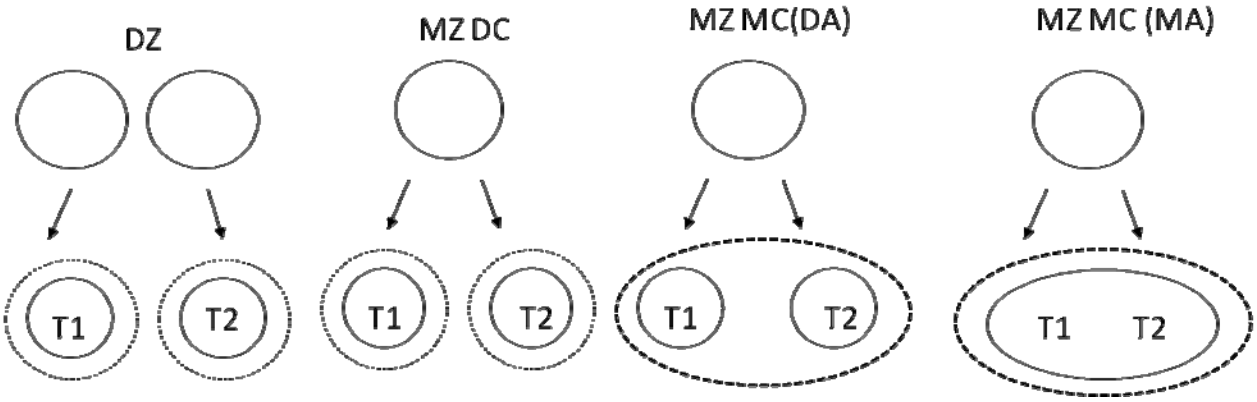

Figure 1.4 Different types of twins according to zygosity, chorionicity and amniotic membranes

DZ=Dizygotic, MZ DC= Monozygotic dichorionic, MZ MC (DA) =Monozygotic monochorionic (diamniotic), MZ MC (MA) =Monozygotic monochoric (monoamniotic)

Thus, twin studies prove to be a valuable tool in epidemiological and psychiatric/psychological research facilitating the implementation of complex genetic models in order to distinguish between genetic and environmental influences and conseqeuntly estimate the size of these influences.

It is of great interest however, that only recently has there been an interest in interpreting traits of child early development within the Developmental Origins of Health and Disease Hypothesis using markers of the intrauterine environment. Given the plasticity of the psychological characteristics that could potentially form personality traits and could act as the precursors of later maladjustive behaviours it is of utmost significance to investigate the early environment of the children and identify the genetic and environmental influences. Even more, as twin studies are now suggesting that genes do not operate apart from the environment in which the individual develops one can raise the question about the usefulness of trying to find associated genes without taking into account the environment. The environment cannot be ignored and the future in twin studies lies in including these environmental influences as well as the influences of the *in utero* environment.

1.5 Objectives of this thesis

- to present the twin methodology based on the East Flanders Prospective Twin Survey (EFPTS) data used in the thesis (Chapter 2)
- to explore the intrauterine environmental factors (birth weight, placenta weight and morphology, umbilical cord knots, cord length and cord insertion) that influence cognitive development (Chapter 3)
- based on the finding of this thesis that knotted umbilical cords are associated with lower IQ scores and supported by previous studies suggesting that the umbilical cord is associated with adverse clinical outcomes I set out to investigate the genetic and environmental influence on the aetiology of the umbilical cord (Chapter 4)
- to present the twin methodology based on the Twins and Multiple Births Association Heritability Study (TAMBAHS) data used in the thesis (Chapter 5)
- as a subproject, to investigate the amount of information people need to read before they participate in research (Chapter 6). This subproject is introduced in Chapter 5.
- to explore further intrauterine factors taking into account maternal pre-pregnancy weight and its association with infant temperamental dimensions (Chapter 7) and behavioural disorders (Chapter 8)
- to discuss the findings (Chapter 9)

1.6 Twin studies of this thesis: Outline

The chapters in this thesis are related to two twin projects, the East Flanders Prospective Twin Survey (EFPTS) and the Twins and Multiple Births Association Heritability Study (TAMBAHS). The chapters will be presented as follows:

Chapter 1 Introduction

Chapter 2 Methods: The East Flanders Prospective Twin Survey (EFPTS)

Chapter 3 Intrauterine environmental factors and cognitive development in young twins: The East Flanders Prospective Twin Survey

Chapter 4 The influence of genetic and environmental factors on the aetiology of the umbilical cord: The East Flanders Prospective Twin Survey

Chapter 5 Methods: The Twins and Multiple Births Association Heritability Study (TAMBAHS)

Chapter 6 An empirical study on the preferred size of the participant information sheet in research

Chapter 7 Maternal pre-pregnancy weight and infant temperament

Chapter 8 Maternal pre-pregnancy weight and behaviour problems

Chapter 9 General Discussion

THE EAST FLANDERS PROSPECTIVE TWIN SURVEY

CHAPTER 2

METHODS: THE EAST FLANDERS PROSPECTIVE TWIN SURVEY (EFPTS)

2.1 General Characteristics

The East Flanders Prospective Twin Survey (EFPTS) is a prospective, population-based registry of multiple births. The EFPTS is a unique registry in terms of the amount of full perinatal data that it contains including placentation and the accurate determination of zygosity (77). Since its establishment in 1964, it has been recording multiple births in the Belgian Province of East Flanders. In Belgium 99% of mothers deliver in maternity wards. In East Flanders there are 19 maternity wards with a total of 14.000 births per year (80). All multiple births are reported to EFPTS within 24 hours after delivery. A trained midwife registers the data within 24 hours after the notification of the delivery. All pairs who meet the World Health Organization criteria for liveborn infants are entered into the registry. Structured questionnaires (delivered by the obstetrician and the pediatrician) are used to collect obstetric and perinatal data. The questionnaires elicit information on birth order, birth weight, mode of conception, fetal presentation, possible abnormalities, ABO and Rh blood groups, health status of the mother before, during and after the delivery and children's health status for as long as they stayed in the maternity ward. The gestational age is calculated as the number of complete weeks of pregnancy.

2.2 Data Collection

2.2.1 Placenta Examination

The placenta examination takes place within 48 hours after delivery by a trained midwife. The fetal membranes are carefully dissected and after removing the membranes and blood clots, the fresh placentas are weighed and measured. The site of the umbilical cord insertion is classified as: central, peripheral, paramarginal, marginal, on the surrounding and on the diving membrane between the two fused placentas. Cord length is measured in centimetres (cm) and the number of umbilical vessels is counted; three vessels are recorded in cases where two arteries and one vein are visible and two vessels, when one artery and one vein are visible. The twisting of the cord, which is essentially the winding of the two arteries around the vein, is also noted and recorded as clockwise if the direction of the winding is right, counter-clockwise if the direction is left, undefined and mixed when there is not a clear direction of the winding. The umbilical cord is also examined for possible knotting. False knots are recorded when there are loose knots on the cord, which are often described as loops or herniations, real knots when the cord is knotted and as no knots (77). A full descriprion of the placenta examination is given by Derom and colleagues (76).

2.2.2 Zygosity determination

Zygosity is determined through sequential analysis based on sex, fetal membranes, umbilical cord blood groups (ABO, Rh, CcDEe, MNSs, Duffy, Kell), placental alkaline phosphatase (81, 82) and since 1985, DNA fingerprints (83, 84). Opposite-sex twins and same-sex twins who have at least one different genetic marker are classified as DZ;

monochorionic (MC) twins are classified as MZ. A probability of monozygosity using a lod-score method (85) is calculated for same-sex dichorionic (DC) twins with the same genetic markers and a zygosity probability of .999 is reached.

2.3 Subsets of twin data within the EFPTS

2.3.1 Twin data (1982-1991)

Sample

All twin pairs (n=867) born between 1st September 1982 and 31st December 1991 were invited to complete the WISC-R (Wechsler Intelligence Scale for Children-Revised) test on three IQ scales, the total, performance and verbal IQ. 204 twin pairs refused to participate, which resulted in a sample of 663 twin pairs (76% participation rate), between the ages of 7 and 15 years old, with a mean age of 10.4 years old. In total, 289 monozygotic (MZ) male twins, 269 monozygotic (MZ) female twins 168 dizygotic (DZ) male twins, 202 dizygotic (DZ) female twins and 370 opposite-sex twins were included in this study. Of these 663 twin pairs, 28 were incomplete pairs (14 MZ twins, 7 DZ liked-sexed and 7 DZ unlike-sexed). For each of these twin pairs, one twin did not have information because of either perinatal death (n=26) or severe mental retardation (n=2). This meant a total sample size of 1,298 twins. A detailed description of the sample is given in Chapter 3.

Materials and measures

The birth weight, the umbilical cord length and type of insertion on the placenta, and five types of placenta morphology were used in order to investigate the relationship between these intrauterine factors and later cogntitive development. A detailed description of all measures and materials used is presented in Chapter 3 under the Methods section.

Statistical Analysis

Random-effects regression models were used where the intercept of each twin pair was modeled as a function of the population plus a unique contribution of the twin pair. Random-effects models provide a flexible regression modeling framework for handling data sampled from clustered population structures, such as the twins within each pair.

Univariate and bivariate genetic modeling were used to estimate the genetic and environmental influence on IQ and the genetic and environmental covariation between IQ and the significant intrauterine factors that correlated with the IQ scales. A detailed description of the statistical analysis is presented in Chapter 3.

2.3.2 Twin data (1964- 2002)

Sample

Between July 1964 and the end of December 2002 EFPTS registered 6.315 twin pairs who met the WHO criteria for live born infants. 205 pairs were excluded because one of both children was stillborn and 120 twin pairs were also excluded due to congenital malformations (86). That resulted in a dataset of 5,990 twin pairs, whose mothers gave their written informed consent according to the local ethics committee guidelines. This

dataset was used for the investigation of the genetic and environmental influence on the aetiology of the umbilical cord (Chapter 4).

Materials and Measures

Measurements of the umbilical cord length, insertion on the placenta, knots, twisting and number of vessels taken by a trained midwife at birth, were used in order to investigate the genetic and environmental influence on the umbilical cord. A detailed description of the variables and their coding used in the analysis is presented in Chapter 4. The measure on cord length was used as a continous variable. For the purposes of the analyses, the six categories of cord insertion defined at birth, were categorized into two groups: 1) central insertion (central, peripheral and para-marginal and 2) peripheral insertion (marginal, membrane septum and membrane peripheral). Membrane septum and membrane peripheral are both cases of velamentous insertion on the dividing membranes between the two fused placentas, and on the surrounding membrane respectively and have both been associated with adverse perinatal outcomes (87, 88). For this reason, the analysis was extended in order to examine the prevalence of velamentous insertions separately from the rest of the categories. The cord knots were categorized as follows: 1) real knots 2) false knots and 3) no knots. Cord twisting was categorized as: 1) clockwise, 2) mixed/undefined and 3) counter-clockwise. The number of cord vessels was categorized as: 1) two vessels and 2) three vessels.

Statistical analysis

Twin similarity for the umbilical cord characteristics was summarised by estimates of concordance. This could be assessed estimating the pairwise concordances separately

for DZ, MZDC and MZMC twins. The ninety-five percent confidence intervals (95% CI) for concordances of the cord characteristics were also calculated. The pairwise concordance is the relative number of the twin pairs in whom the trait under study is observed in both twins in a pair and it is calculated with the formula C/C+D, in which C is the number of concordant pairs and D is the number of discordant pairs. Intra-pair twin correlations in MZ and DZ pairs were also calculated in order to get a first estimate of the genetic and environmental influence on the umbilical cord characteristics. Because some of the phenotypes used in the analysis were categorical the Spearman's correlation coefficient (89) was used. For the continuous variable, cord length, the Pearson's correlation coefficient was used (90). For the genetic analysis, a threshold model assuming an underlying liability scale was used and the analysis was further extended in order to allow for the investigation of sex differences. A detailed description of the variables used for the statistical analysis is presented in Chapter 4 under the Methods section.

CHAPTER 3

INTRAUTERINE ENVIRONMENT AND COGNITIVE DEVELOPMENT IN YOUNG TWINS

3.1 Introduction

The intrauterine environment is an important factor in the development of many diseases and adult health. Factors considered particularly important are birth weight, chorionicity/shared placenta and umbilical cord abnormalities (91-94). An important developmental outcome is intelligence. Intelligence can be thought of as reflecting the ability for reasoning, problem solving and concept understanding (95). Childhood intelligence is predictive of educational attainment and later socioeconomic status which in turn have significant social and public health implications (96-98). Given the impact of intelligence on the later life course it is important to understand the underlying mechanisms behind variation in cognitive functioning.

Intellectual performance is influenced by a mixture of genetic and environmental effects (32, 99-102). A review (103) and meta analysis of 30 studies conducted in twins concluded that 44% of the variance in IQ scores is explained by genetic factors. However, much higher heritability estimates (~85%) have also been reported in familial studies (104). A meta-analysis examining the heritability of IQ have consistently found that genetic factors account for about 50% of the IQ variance (105), but in single studies heritability estimates range from 20-80% (27, 95).

Intellectual development, including cognitive deficits such as learning disorders and delays in language have been linked to low birth weight (27-29, 106). Boomsma and her colleagues (33) have shown that genetic factors may mediate the relationship between low birth weight and intelligence. However, some studies suggest that environment *in utero* may be more important in explaining the relationship with IQ, with these factors accounting for up to 20% of the variation in intelligence (34).

Several intrauterine factors have been implicated in poor outcomes including smaller placentas increasing the risk of limited fetal development (107). Chorionicity is likely to also play an important role in intrauterine twin growth (108-110). Twins who share the same placenta or chorion are known to have compromised intrauterine growth (111-113). Moreover, the site of the umbilical cord insertion on the placenta can restrict fetal development and could cause later abnormalities, such as lower birth weight (86).

Umbilical cord abnormalities are a third marker of poor intrauterine environment. Adverse perinatal outcomes have been reported with both abnormally long and abnormally short umbilical cords (114-116). Infants with excessively long umbilical cords have a significantly higher likelihood of brain imaging abnormalities and abnormal neurological follow-up in later life (117, 118). It has been reported that decreased cord length correlates with decreased IQ and a greater frequency of motor abnormalities and Down syndrome (18, 24). Less common, but with potentially devastating consequences, is the occurrence of cord knotting. A knot can constrict the blood vessels and lead to fetal death. Cord knots appear to be associated with fetal growth and compromise the communication between the mother and the fetus causing subsequent obstructions in nutrients supply (119). Cognitive development is influenced by the nutritional status in utero (120) and anything that can constrict the normal flow of nutrients maybe be potentially influential to later outcomes. The full mechanism by which umbilical cord abnormalities produce intrauterine fetal growth restriction is not known and it is not clear to what degree they may affect later cognitive performance.

Individual differences between peoples' IQ might be attributable to the intrauterine environment, genetic factors and the subsequent family environment (121, 122). An important question remains to be answered as to whether these individual differences seen between people's IQ have their origin in the intrauterine environment. The intrauterine environment has not yet proved to be as influential as the infant's genetic endowment regarding later cognitive development, but nevertheless is of considerable importance (123). Previous research has shown that the association between birth weight and IQ could be explained by genetic factors (33, 112), while others suggest that genetic factors do not account for the relationship between these two (113).

With this study, the effect of genetics and intrauterine environment on intelligence using a genetically sensitive design is examined. More specifically, the relationship between IQ and the following measures of intrauterine environment: birth weight, placental weight, placental morphology, cord knots, cord length and cord insertion was investigated.

3.2 Methods

3.2.1 Sample

The East Flanders Prospective Twin Survey (EFPTS) has recorded multiple births in the province of East Flanders (Belgium) since 1964. All twin pairs (n=867) born between 1st September 1982 and 31st December 1991 were invited to complete the WISC-R (Wechsler Intelligence Scale for Children-Revised) test on three IQ scales, the total, performance and verbal IQ. Of these, 204 twin pairs refused to participate. That resulted in a sample of 663 twin pairs (76% participation rate), between the ages of

seven and 15 years old, with a mean age of 10.4 years old, which were included in this study: 289 monozygotic (MZ) male twins, 269 monozygotic (MZ) female twins 168 dizygotic (DZ) male twins, 202 dizygotic (DZ) female twins and 370 unlike-sex twins. Of these 663 twin pairs, 28 were incomplete pairs (14 MZ twins, seven DZ liked-sexed and seven DZ unlike-sexed). For each of these twin pairs, one twin did not have information because of either perinatal death (n=26) or severe mental retardation (n=2). This meant a total sample size of 1,298 twins. There were no differences between the twins who participated in this study and the ones who refused to participate in terms of gestational age ($p=0.94$) and birth weight ($p=0.86$). However, in the final sample used in this study more MZ twins than DZ twins ($p<0.01$) participated in this study and thus unlike-sex twins were underrepresented in the final sample ($p<0.01$). The parents of the twins gave their written informed consent according to the local ethics committee guidelines.

The zygosity of the twins was determined by sequential sex, placentation, blood groups, and examination of five highly polymorphic DNA markers. Unlike-sex twins were classed as dizygotic as were same-sex twins with at least one different genetic marker; monochorionic twins were classified as monozygotic. For all the same-sex dichorionic twins with the same genetic markers a probability of monozygosity was calculated using a lod-score method. After DNA fingerprinting, a probability of monozygosity of 0.999 was reached (124).

3.2.2. Measures

All twins completed the Wechsler Intelligence Scale for Children-Revised (WISC-R). This consists of six verbal and six performance subscales and has been validated for use in this population (37). The verbal subscales are Information (INF), Similarities (SIM), Arithmetic (ARI), Vocabulary (VOC), Comprehension (COM) and Digit Span (DS). The performance subscales are Picture Completion (PC), Picture Arrangement (PA), Block Design (BD), Object Assembly (OA), Coding (COD) and Mazes (MAZ). The scores on the subscales are standardized for age and added up to Verbal (VIQ), Performance (PIQ) and Total Intelligence Quotients (TIQ). In this study the total scores of the subscales and the total intelligence quotient (IQ) score were analysed. The test was administered simultaneously to the twins by two different trained research workers in two different rooms made available for the researchers at the University of Ghent in Belgium.

The type of the placenta was determined within 48 hours of delivery by a trained midwife at the same time as chorion type and the total weight of the placental mass was recorded based on a standardized protocol (76). The cord insertion was recorded as central, eccentric, paramarginal, marginal, on the surrounding membrane, or on the dividing membrane. The umbilical cord length and knots were also noted and recorded at delivery. Birth weight was obtained from the obstetric notes and recorded within 24 hours of delivery and gestational age was calculated as the number of complete weeks of pregnancy.

Based on previous literature the umbilical cord length was categorized as short (0-40cm), average (41-69cm) and long (70-100cm) while it was also analysed as a continuous trait.

Cord knots were categorized as: 1) knots and 2) no knots. Tight and loose knots of the umbilical cord were combined in the first category. According to the site of the cord insertion the six categories distinguished were divided into two groups: (1) central insertion (central, paracentral, paramarginal) and (2) peripheral insertion (marginal, membrane septum and membrane peripheral). There were five categories for the placenta morphology: (1) two separate placentas dichorionic diamniotic (DCDA) (2) two placentas connected with membranes (DCDA) (3) one fused placenta (DCDA) (4) one placenta monochorionic diamniotic (MCDA) and (5) one placenta monochorionic monoamniotic (MCMA).

3.3 Statistical analyses

3.3.1 Regression analyses

A random-effects regression model was used, where the intercept of each twin pair was modeled as a function of the population intercept plus a unique contribution of the twin pair. Based on these models, expected IQ scores and standard errors for each level/category of the markers for intrauterine nutrition were computed. Potential covariates, which have been previously suggested to have an influence on the relationship between the intrauterine factors and IQ were checked, and it was found that the age of the twins at testing and the parental educational level did not have a significant effect on the relationship between the intrauterine factors under study and the

IQ scores. The twins' gestational age and gender were adjusted in the analysis. Only the significant associations from the regression analysis were further examined in bivariate genetic analyses. The analysis was performed using the statistical software package STATA 10 (125).

3.3.2 Bivariate genetic analyses

Bivariate genetic analysis was used to examine the relationship between the measures of IQ (Total, Performance and Verbal) and those intrauterine factors which significantly associated with IQ (cord knots and birth weight). Variance decomposition was applied leading to an estimate of the correlation between the genetic, common environmental and non shared environmental components between the two phenotypes.

To estimate how much of the phenotypic correlation between IQ and cord knots, and between IQ and birth weight was due to overlapping genetic and environmental factors, the genetic and environmental correlations were weighted by the square root of the heritabilities and the environmental influence on the traits and divided by the phenotypic correlation (126). Based on the MZ and DZ ratios of the univariate correlations of the IQ scales, cord knots and birth weight, ACE factors were modelled in the bivariate analyses. Full models are only displayed in Table 3.4.

To enable the fitting of the models for the examination of the correlation between cord knots and IQ, the continuous IQ scores were used in the models as quintiles using threshold liability models. The gestational age and gender in the model of the means (IQ – birth weight) and thresholds (IQ – cord knots) were adjusted.

The figure below shows a bivariate genetic model. The correlation between birth weight and IQ has been taken as an example. The object of the bivariate model in general is to decompose the correlation between two phenotypes into A, C and E components. With this model we expect that within twin-cross phenotype correlations will be similar for MZ and DZ twins. The cross-twin cross-phenotype correlations will differ. Based on that, we follow the same rules to estimate a^2, c^2 and e^2 as was described for the univariate genetic model in the Introduction of the thesis.

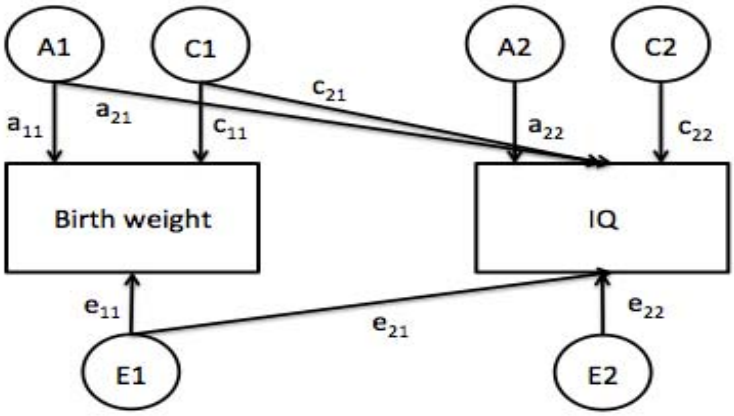

Figure 3.1 Path diagram for the bivariate twin model

3.4 Results

Table 3.1 shows descriptive characteristics for the entire twin sample on the variables used in the analyses. Monozygotic twins had significantly more peripheral cord insertions than dizygotic twins ($p<0.001$). Dizygotic twins had significantly more knots ($p=0.04$) more fused placentas ($p<0.001$) higher placental weight ($p<0.01$) and higher

birth weight ($p<0.01$) than monozygotic twins. There were no significant differences between MZ and DZ twins in terms of gestational age and the IQ scores. Moreover, IQ scores are just above 100 indicating that twins IQ scores can be compared to general population's mean IQ scores.

3.4.1 Regression Analyses

A significant effect of birth weight was seen for all IQ scales. Twins with higher birth weight performed better in the IQ scales: For each increase of 100gr in birth weight, there was a corresponding increase of 0.38 ($p<0.01$) in the total IQ, an increase of 0.43 ($p<0.01$) in the performance IQ and an increase of 0.25 points ($p=0.02$) in the verbal IQ. When we performed the analysis excluding the twins with low birth weight (<1500gr) the effect of birth weight on IQ was still significant ($p<0.01$).

Cord knotting had a significant effect on the total and verbal IQ scores. Twins with knots had lower (of 1.92 points, $p= 0.02$) total IQ and verbal IQ score (of 1.70, $p=0.04$) compared to twins with no knots. Placenta weight and morphology did not have a statistically significant effect on IQ scores; neither did the other umbilical cord features. When the cord length was used in the analyses as a continuous trait the results suggested that there was not a significant effect on the IQ scores for the three subscales (for total IQ, $p=0.86$, $b=0.007$, $SE=0.04$; for performance IQ, $p=0.88$, $b=-0.006$, $SE=0.04$; for verbal IQ, $p=0.66$, $b=0.02.74$, $SE=0.04$). More specifically, these results mean that with every centimetre increase of cord length there was an increase of 0.007 and 0.02 points of total and verbal IQ respectively and a decrease of 0.006 points of performance IQ (results are not shown in table).

The expected means of the IQ scales for each intrauterine marker are displayed in Table 3.2.

3.4.2 Bivariate Genetic Analyses

The significant association between cord knots and the three IQ scales and between birth weight and the three IQ scales were further investigated in six bivariate ACE models. The genetic and environmental components of cord knots, birth weight and the IQ scales and 95% confidence intervals are displayed in Table 3.4. In addition, the extent to which the A, C and E factors are correlated across the two traits are given in the last three columns. To estimate how much of the correlation between the two phenotypes in each analyses was due to genetic and environmental factors, the genetic and environmental correlations, respectively, were weighted by the square root of the heritabilities and environmental loading of the IQ scales, cord knots and birth weight (i.e., the contribution of the non- shared environment to the phenotypic correlation between birth weight and total IQ is $\sqrt{.56} \times .08 \times \sqrt{.18} = .05$, which is 17% (.025/.15). In like manner, the contributions due to genetic and shared environment in all bivariate analyses of knots and IQ, and birth weight and IQ, can be calculated. The phenotypic correlations and the part explained by genetic, environmental factors are displayed in Table 3.3.

Table 3.4 shows the results of the 6 bivariate models. The last three columns present the correlations between the phenotypes. For the three IQ scales and birth weight a negative common environment correlation between the two traits is observed. The genetic factor (A) could be important in explaining the correlation between the two but with wide

confidence intervals and A not being an important factor for birth weight but only for IQ the results should be interpreted with caution. However, the non-shared environment may explain moderately the association between the two but in genetic analysis it is difficult to completely distinguish the genetic effects from the other factors. For the other set of analyses between the three IQ scales and cord knots, a negative genetic correlation is observed. Common environmental factor is a significant factor but because it is not an important factor for IQ a potential model would suggest that A explains all familial correlation. However, genetic effects cannot solely contribute to the explanation of the association between the two phenotypes and the non-shared environment should be taken into account.

3.5 Discussion

The results of this study suggest that intelligence is highly heritable, with estimates ranging from 70 to 82% for all IQ scales on a continuous scale. These findings are consistent with previous research (99, 104, 127). Previous studies have also suggested that early malnutrition has been associated with later developmental damage (123, 128, 129). Inadequate nutrition in utero is important as the critical period of brain growth is before birth and during early postnatal life (130). The findings of this study support previous research suggesting that birth weight, as a marker of intrauterine environment, is associated with lower IQ scores. The negative effect of low birth weight on intellectual development is well documented in literature (27, 28, 31). These results suggest that there is an association between birth weight and intelligence. For the whole sample of twin pairs, twins with an increase of 100g in birth weight had a corresponding increase of 0.38 points in total IQ, 0.43 in performance IQ and 0.25 points in verbal IQ

respectively. As suggested by the bivariate analysis results, with non-shared environmental factors accounting for the majority of the correlation between birth weight and performance and verbal IQ respectively it can be speculated that situations in utero experienced in a unique way by the twin individuals might affect later cognitive development.

Our results are similar to those previously reported. Richards and his colleagues (131) found an association between birth weight and cognitive ability at age eight across the normal range of birth weight in the British 1946 birth cohort. In addition, Breslau and his colleagues (132) reported that birth weight was associated with IQ at age six in a sample of several hundred low and normal birth weight children and that the association extended into the range of normal birth weights. From a clinical point of view, the effect of even 100g difference in birth weight on IQ, found in our study, may seem not very important. At the population level, however, many children are born with birth weights above or around 2,500g than are born with low birth weights. Small shifts in the distribution of birth weight among normal birth weight babies may have greater impact on the population distribution of intelligence than larger shifts in the percentage of babies born at low birth weight. It may therefore be important to identify the sources of variation in birth weight that are both modifiable by prevention and linked to cognitive ability.

Heritability estimates of birth weight were moderate (20% of variance explained by genes) which is in agreement with previous studies (133, 134). Van Baal and Boomsma (135) offered a plausible explanation that distinguishes the low influence from fetal genes. They suggested that monozygotic, and especially monochorionic twins who share the same chorion, may compete against each other for nutrients and therefore

exhibiting differences in birth weight which are not predicted by a genetic model. However, the variance of birth weight explained by genes reported in this study is not negligible and it can be speculated that only part of the relationship between birth weight and total IQ can be genetically mediated. Boomsma and her colleagues (33) showed an association of intrapair differences in birth weight and IQ. This association was positive for DZ twins and not for MZ twins at ages seven and 10, suggesting that genetics may mediate this relationship. Nevertheless, Petersen and his colleagues (136) found no association between these two phenotypes.

The findings of this study suggest that genetic factors may influence the relationship between birth weight and IQ, although the wide confidence intervals (CI: 0.03-1.00) of the correlation between birth weight and IQ suggest a more conservative interpretation of these results, which leaves us to consider other potential explanations of this association.

There has been some speculation about how the association between birth weight and cognitive function might occur (137). There is evidence indicating that insulin-like growth factors (IGFs) play a critical role in determining overall body growth in addition to contributing to local tissue regulation (138). IGFs are peptides that regulate the growth, metabolism, survival and differentiation of cells and are regulated by growth hormone. It has been suggested that early in life IGFs and growth hormone are important for the development of parts of the brain which are responsible for learning and memory, which could explain the association between body size and cognitive functions (139).

The results for the effects of cord knots on IQ suggest that the twins with knots have statistically significant lower total (of 1.92 points) and verbal IQ (of 1.70) scores compared to twins with no knots. The performance IQ was also lower but not statistically different for twins with knots compared to twins with no knots. To the best of my knowledge, this is the first study to report on the significance of cord knotting regarding cognitive development. The results from the bivariate model suggest that non-shared prenatal influences may explain the relationship between knotting and IQ. It is plausible to think that exposure to different intrauterine situations affect knotting and eventually IQ development. For the formation of the cord knots, it can be speculated that there may be a stage where the helical cord makes a loop giving rise to the formation of knots and eventually have an adverse impact on fetal growth and later development. That stage is possibly during pregnancy and within the intrauterine environment while the fetus is still growing in utero. Sornes (140), trying to explain the mechanism by which knots cause growth limitations has shown than that it is more likely that there is a stage during pregnancy, within the intrauterine environment and while the fetus is moving randomly within a confined space, which gives rise to the formation of knots. The results of this study suggest that a sizeable proportion of the variance of knots can be attributed to genetic factors. However, the results of the bivariate analysis suggested that non-shared environment influences the relationship between cord knots and IQ scores, even though both phenotypes are highly heritable. Genes, often, express themselves through the environment. The first environment of the twins is the uterus where the parental genes and the genes of each twin operate. However, in the intrauterine environment the genetic influence is likely to be shared, which means that it will impact on both twins equally. The intrauterine shared

environment may alter the way the knotting is demonstrated in the uterus, and therefore, the consequences may be unique to individual twin members. The non-shared correlation between knotting and IQ may suggest that the twins or the parental genetic influence can affect their own intrauterine environment and even in the very early stages of life twins can experience unique environmental influences that may affect their later development. The non-shared environmental factors influencing IQ might as well relate to differences in activities in later life that foster cognitive growth (141).

In this study, other cord features did not seem to be associated with cognitive development. The site of the umbilical cord insertion, whether central or marginal to the placenta, the placenta weight and its morphology did not have any effect on IQ scores.

Neither was a significant relationship observed between cord length and IQ when cord length was analyzed as continuous or categorical trait, which is contrary to previous research suggesting there is a link (18).

In this study specific markers of the intrauterine environment were examined that may be associated with later cognitive development based on the hypothesis that a favourable intrauterine environment is an indicator of optimal fetal and later post-natal growth. The major sources of variance influencing birth weight, knotting and IQ were different, with non-shared environment showing a larger influence on cord knotting, shared and non-shared environment influencing birth weight and additive genes affecting IQ to a greater extent. Generally, this results suggest that aspects of the non-

shared prenatal environment account for the correlation between birth weight and performance and verbal IQ and the correlation between knotting and IQ.

3.5.1 Conclusion

To summarize, a contribution of genetic factors may explain the observed relationship between birth weight and total IQ. The knowledge that genes may contribute to the relationship between birth weight and IQ can guide research for identification of specific genes. A thorough investigation of this relationship may require the inclusion of maternal lifestyle information. Since this study is the first to report on the influence of knotting on cognitive development, it can provide a valuable insight for a thorough exploration of the mechanisms responsible for the formation of knots in the intrauterine environment and the effect of the genetic and environmental factors on that, which may eventually influence the development of IQ.

Table 3.1 Phenotypic characteristics of the twins sample according to their zygosity

	MZ twins		DZ twins		p-values
General Characteristics	**n**	**%**	**n**	%	
Gender					
MM	289	51.8	168	22.7	
FF	269	48.2	202	27.3	
MF	-	-	370	50	
Cord characteristics					
Cord Insertion					
Central	387	69.3	678	91.6	<0.001
Peripheral	159	28.5	56	7.8	
Cord Knots					
Knots	146	26.2	235	31.7	0.04
No Knots	404	72.4	492	66.5	
Placenta morphology					
2 separate placentas	22	3.9	101	13.6	
2 placentas connected with membranes (DCDA)	55	10	262	35.4	
1 fused placenta (DCDA)	119	21.3	371	50.1	<0.001
1 placenta (MCDA)	351	62.9	0	0	
1 placenta (MCMA)	9	1.6	0	0	
	Mean	**SD**	**Mean**	**SD**	
Cord length (cm)	35.2	10.8	34.9	11.3	0.68
Birth weight (g)	2,427	527	2,511	505	<0.01
Placental weight (g)	726	162	748	145	<0.01
Gestational age (weeks)	36.5	2.7	36.6	2.5	0.71
Intelligence scores (IQ)					
Total IQ	101.7	14.6	102.9	14.9	0.15
Performance	100.3	15.4	101.5	15.4	0.17
Verbal	102.7	14.1	103.7	14.7	0.21

MZ,Monozygotic; DZ, Dizygotic; MM, Male male; FF, Female female; MF, Male female; DCDA, Dichorionic Diamniotic; MC,Monochorionic; MA, Monoamniotic;

Table 3.2 Expected mean IQ scores for each intrauterine factor

	Total IQ			Performance IQ			Verbal IQ		
	Mean	St. Error	P	Mean	St. Error	P	Mean	St. Error	P
Birth weight									
-300gr	101.22	0.63		99.61	0.66		102.51	0.63	
-200gr	101.60	0.59		100.04	0.60		102.76	0.58	
-100gr	101.98	0.55		100.47	0.57		103.01	0.55	
Mean -2475gr-	102.35	0.54	<0.01	100.90	0.55	<0.01	103.26	0.54	0.02
+100gr	102.73	0.55		101.33	0.56		103.51	0.55	
+200gr	103.10	0.58		101.76	0.60		103.76	0.57	
+300gr	103.48	0.63		102.19	0.65		104.01	0.62	
Placental Weight									
-200gr	102.11	0.95		101.23	0.96		102.54	0.93	
-100gr	102.20	0.67		101.04	0.68		102.87	0.66	
Mean - 737gr-	102.29	0.54	0.81	100.86	0.56	0.64	103.20	0.54	0.39
+100gr	102.38	0.67		100.68	0.68		103.54	0.66	
+200gr	102.47	0.95		100.50	0.97		103.87	0.94	
Morphology of placenta									
2 separate placentas	103.42	1.17		102.63	1.19		103.62	1.15	
2 placentas connected with membranes(DCDA)	102.84	0.72		101.69	0.73		103.42	0.71	
1fused plac (DCDA)	102.26	0.55	0.30	100.76	0.56	0.10	103.21	0.54	0.71
1 placenta(MCDA)	101.67	0.84		99.83	0.86		103.01	0.83	
1 placenta (MCMA)	101.09	1.32		98.90	1.34		102.81	1.30	
Umbilical cord									
Cord knots									
Knots	101.02	0.80	0.02	99.82	0.85	0.10	102.09	0.79	0.04
No knots	102.90	0.59		101.32	0.61		103.77	0.58	
Cord Insertion									
Central	102.27	0.56	0.74	100.89	0.57	0.99	103.19	0.56	0.68
Peripheral	102.59	0.97		100.88	1.04		103.57	0.96	
Cord Length									
Short	102.40	0.59		101.03	0.61		103.20	0.58	
Average	102.28	0.79	0.88	100.59	0.84	0.62	103.51	0.78	0.69
Long	102.16	1.49		100.14	1.62		103.83	1.46	

*Adjusted for gestational age and gender

Table 3.3 Total phenotypic correlations and part of correlations explained by genetic, common and non-shared environment factors

		Total Phenotypic correlation	r phenotype due to A	r phenotype due to C	r phenotype due to E
Cord Knots	IQ	-0.37	-.50	0.09	0.04
	PIQ	-0.10	-0.11	0.14	0.07
	VIQ	-0.22	-0.35	0.08	0.05
Birth Weight	IQ	-0.15	0.15	-0.02	0.02
	PIQ	-0.13	0.14	-0.04	0.03
	VIQ	-0.02	0.08	-0.08	0.02

r phenotype due to A : phenotypic correlation between the two factors explained by genetic factors
r phenotype due to C : phenotypic correlation between the two factors explained by common environment factors
r phenotype due to E : phenotypic correlation between the two factors explained by non-shared environment factors

Table 3.4 Estimates and the 95% confidence intervals for the bivariate model for cord knots, birth weight and IQ scales (the last used as quintiles)

		AI (95% CI)	CI (95% CI)	EI (95% CI)	A2 (95% CI)	C2 (95% CI)	E2 (95% CI)	rA (95%CI)	rC(95%CI)	rE(95%CI)
Cord Features										
	IQ	0.41 (0.00-0.69)	0.14 (0.00-0.49)	0.45 (0.30-0.65)	0.67 (0.49-0.86)	0.18 (0.00-0.34)	0.15 (0.12-0.22)	-0.03 (-0.67-1.00)	0.61 (-1.00-1.00)	0.17 (-0.08 -0.41)
Cord Knots	PIQ	0.33 (0.00-0.65)	0.20 (0.00-0.50)	0.47 (0.31-0.66)	0.64 (0.44-0.78)	0.10 (0.00-0.27)	0.26 (0.21-0.33)	-0.24 (-1.00-0.20)	1.0 (-1.00 -1.00)	0.20 (-0.02-0.41)
	VIQ	0.41 (0.00-0.68)	0.14 (0.00-0.49)	0.45 (0.30-0.65)	0.60 (0.42-0.79)	0.23 (0.05-0.39)	0.17 (0.14-0.22)	-0.14 (-1.00-1.00)	0.74 (-1.00-1.00)	0.19 (-0.05-0.42)
Birth Weight	IQ	0.04 (0.00-0.29)	0.40 (0.19-0.48)	0.56 (0.47-0.63)	0.74 (0.58-0.84)	0.08 (0.00-0.24)	0.18 (0.15-0.22)	0.85 (0.03-1.00)	-0.55 (-1.00 -0.14)	0.08 (-0.04-0.20)
	PIQ	0.04 (0.00-0.29)	0.40 (0.20-0.48)	0.56 (0.47-0.63)	0.70 (0.51-0.77)	0.03 (0.00-0.20)	0.27 (0.22-0.32)	0.85 (-0.24-1.00)	-0.86 (-1.00-1.00)	0.08 (-0.03-0.20)
	VIQ	0.01 (0.00-0.29)	0.42 (0.19-0.49)	0.56 (0.47-0.63)	0.67 (0.52-0.83)	0.14 (0.00-0.29)	0.19 (0.15-0.23)	0.99 (-0.47-1.00)	-0.32 (-1.00-0.21)	0.07 (-0.05-0.19)

IQ, Intelligence Quotient; PIQ, Performance IQ; VIQ, Verbal IQ
AI, CI, EI; Additive genetic, common and non-shared variance for cord knots and birth weight
A2, C2, E2; Additive genetic, common and non-shared variance for total, Performance IQ and Verbal IQ
r A, r C, r E: Additive genetic, common and non-shared correlations between IQ scales and cord knots and birth weight respectively
^The A, C,E, estimates for the IQ scales may be slightly different from the continuous analyses, because they are used as quintiles in the bivariate analyses with cord knots

*Adjusted for gestational age and gender

CHAPTER 4

THE INFLUENCE OF GENETIC AND ENVIRONMENTAL FACTORS ON THE AETIOLOGY OF THE UMBILICAL CORD

4.1 Introduction

The umbilical cord is the principal connection between the fetus and the placenta, providing the nutrients, oxygen and fluids necessary for life *in utero*. The cord and its constituent tissues, an outer layer of amnion, porous Wharton's jelly, two arteries and one vein, are designed to provide and maintain the blood flow to the developing fetus (22).

Although the umbilical cord is one of the most vital components of the fetal anatomy, it is still one of the least studied fetal structures. Given the fact that the umbilical cord is vulnerable to a number of abnormalities which may occur during pregnancy, labor or delivery, it is important to investigate the underlying mechanisms of the development of cord morphology and possible pathologies associated with it, since this may provide an insight into fetal growth within the intrauterine environment and its impact on later life. Some of the morphological aspects of the umbilical cord, such as its length, knots, insertion to the placenta, number of vessels and twisting have been associated with pathological outcomes (24, 25, 115, 142, 143). At term, the typical umbilical cord length is 55 to 60 cm (24). Adverse outcomes have been reported with both abnormally long (70 to 80 cm) and abnormally short cords (30 to 40 cm) (114). Long or short cords can be the cause of hematomas and thrombosis of cord vessels and the surface of the placenta thus causing fetal hypoxia, damage of the central nervous system or even fetal death (26). Infants with excessively long umbilical cords have a significantly higher likelihood of brain imaging abnormalities and abnormal neurological follow-up in later life (117). Results from a recent case report and literature review support earlier findings where excessively long cords have been associated with fetal loss, long-term

neurological complications and fetal thrombotic vasculopathy (118). In a case control study looking at infants with short cords and infants without a diagnosis of a short cord, Krakowiak and his colleagues found that infants with short cords were more likely to have congenital malformations, be small for gestational age and have more adverse outcomes, such as fetal distress and death within the first year of life (144). Moreover, it has been reported that decreased cord length correlates with depressed intelligence quotient values and psychomotor abnormality (18).

Cord knotting may potentially have devastating consequences. In a population study of 69.000 singletons, knots were associated with grand multiparity, chronic hypertension, hydramnios, cord prolapse and cord around the neck (145). Moreover, a four-fold higher rate of antepartum fetal death was associated with these singletons. Airas and his colleagues reported that knots were associated with previous miscarriages, obesity, long cord and maternal anaemia (146).

Variations in the site of the cord insertion to the fetus and the placenta and its relationship with later development have also been reported. The site of the cord insertion to the placenta is associated with later developmental abnormalities and growth restrictions (86, 107, 147). These are generally categorized as placental insertions in the centre, off centre, on the edge, or in the membranes. An insertion to the centre of the placenta is more favourable compared to a marginal one which appears towards the edge of the placenta (148). The marginal insertion, which is most commonly referred to as velamentous insertion, is the insertion into the membranes. This type of insertion is often associated with the worst outcomes.

The veins traverse the membranes before they come together into the umbilical cord and the cord inserts into the chorioamniotic membranes rather than on the placental mass. The incidence of this condition is 1.1% in singletons and 8.7% in twins. Moreover, the prevalence of velamentous cord insertion in monochorionic and dichorionic twin placentas is higher than in singletons placentas (12% and 7% vs 2%, respectively) (149). The presence of velamentous insertion is associated with an increased risk of birth weight discordance, especially in monochorionic twins (150) and fetal vessel thrombi (151).

The intrauterine environment, where the twins develop, has been suggested to have an important influence on later outcomes and is associated with the site and type of the insertion on the placenta. Twins who share the same chorion (Monochorionic-MC) due to limited space may compete against each other for resources provided from the mother, compared to twins who have different chorions (Dichorionic-DC). MC twins are prone to adverse outcomes, which have often been attributed to mainly complications caused by placental vascular anastomoses (78, 79) Very often, perinatal mortality rate is higher in MC rather than DC twins (152-154) while the incidence of birth weight discordance is higher in MC twins (155).

Within the cord there are two arteries and one vein, thus three vessels in total. The two arteries send blood with waste products from the fetus to placenta and umbilical vein sends oxygen and nutrient-enriched blood to the fetus from the placenta. Often only two vessels are grossly visible; one artery and one vein. Single umbilical artery occurs in about 5% of cords in at least one twin and occurs more often in fetal demise than in live

births (156). Of infants with a single umbilical artery, 20% or more are reported to have associated fetal anomalies including cardiovascular abnormalities (156), a variety of renal defects and multiple anomaly syndromes (157, 158).

Counterclockwise/left or clockwise/right cord twists can be seen as early as the 6th week and are well established by the 9th week of development. Results from a case report by Herman and colleagues (159) suggested that large number of twists of the cord causing torsion of the length of the cord was found in two cases of intrauterine fetal death. Hypertwisted cords have also been associated with intrauterine growth restriction, non-reassuring fetal tracing and increased rate of emergency Ceasarean section (143). Obstetric outcomes and findings from placental examination have revealed that placenta previa, third trimester bleeding and single umbilical artery (160, 161) are more likely to occur in cases with a clockwise twist compared to a counterclockwise one.

No study had so far investigated the relative contributions of genetic and environmental influences on the different characteristics of the umbilical cord. Specifically, with this study the genetic and environmental aetiology of cord length, insertion, knots, twisting and number of vessels is examined.

4.2 Methods

4.2.1 Sample

The East Flanders Prospective Twin Survey (EFPTS) has recorded multiple births in the province of East Flanders (Belgium) since 1964. From an initial sample of 6,315 twin pairs registered with EFPTS from 1964 to 2002, 5,990 twin pairs were selected for these

analyses. From the initial sample, 205 twin pairs were excluded due to one or two twins being stillborn and 120 twin pairs were excluded due to congenital malformations. That resulted in a total of 11,980 twins included in the final analyses. Placentas were collected at birth and examined by a trained midwife within 48 hours after delivery. Fetal membranes were carefully dissected as fully described by Derom et al. (82). All parents of the twins gave their written informed consent according to the local ethics committee guidelines.

Zygosity was determined by sequential sex, placentation, blood groups and since 1985, by DNA fingerprinting. Opposite-sex twins and same-sex twins with at least one different genetic marker were classified as dizygotic; monochorionic twins were classified as monozygotic. For all the same-sex dichorionic twins a probability of monozygosity was calculated. After DNA fingerprinting, a probability of monozygosity of 0.999 was reached (124).

4.2.2 Measures

The umbilical cord was examined by a trained midwife at birth. For this analysis, cord length was treated as a continuous variable. The six categories of cord insertions were divided into two groups: 1) central insertion (central, peripheral and paramarginal and 2) peripheral insertion (marginal, membrane septum and membrane peripheral). As velamentous insertions have been previously associated with poor perinatal outcomes and quite often with fetal hemorrhage (87, 88) the analysis was repeated after the categories central, peripheral, para-marginal and marginal were combined into the “non velamentous” category and the categories membrane septum and membrane peripheral, which are cases of velamentous insertions on the cord, were recorded into the second

category, the “velamentous” one. Cord knots were categorized as 1) real knots, 2) false knots and 3) no knots. Since knotting is more common in monoamniotic monochorionic (MoMo) twins (146), MoMo twins with cord knots were excluded from the analysis.

Cord twisting was categorized as 1) clockwise, 2) mixed/undefined and 3) counter-clockwise. The number of cord vessels was categorized as: 1) two vessels and 2) three vessels. The ordering of the knots and twisting was based on previous literature suggesting that more adverse outcomes are associated with real knots (140) or clockwise twisting (160, 161), while the frequency of problems diminish in the other categories.

4.3 Statistical analysis

4.3.1 Univariate Genetic Analysis

Intra-pair twin correlations and pairwise concordance rates were calculated to examine the genetic and environmental influences on these phenotypes. The correlations of the categorical variables were estimated using the Spearman’s coefficient (89) and for the correlations of the continuous variable cord length the Pearson’s coefficient was used (90).Threshold models for cord knots, insertion, number of vessels, and twisting were used assuming an underlying liability scale adjusting to a threshold model of inheritance (72).

Variance decomposition was applied to the liability for cord knots, insertion, number of vessels and twisting, leading to an estimate of the heritability of liability (69). A maximum likelihood fit function was calculated based on raw data using the statistical

software package Mx (71). The estimates of the heritability of the liability are presented with 95% confidence intervals (95% CI) and goodness of fit statistics for 3 models: a full ACE model, in which the phenotypic variance is explained by genetic (A), common environmental (C) and non-shared environmental factors (E), a model in which all variance was attributed to genetic and non-shared environmental factors (AE) and a model in which all variance was explained by non-shared and common environmental factors (CE). Reduced models were estimated by removing one of the parameters at a time and re-running the model. The goodness of fit of the reduced models was compared to the full model to assess whether they represented a better explanation of the data using the likelihood ratio χ^2 test and the Akaike Information Criterion (AIC). The models were assessed by examining the decrease in the fit of the model; if a parameter could be dropped without a significant decrease in fit then on the grounds of parsimony the reduced model was accepted as the best fitting model (71).

4.3.2 Gender-effect genetic models

The analysis was further extended in order to examine possible differences in the aetiology of the variations in cord morphology between males and females. A general-effects (heterogeneity) gender-limitation model, a common-effects gender-limitation model and a gender-homogeneity model were fitted (71). By comparing a general heterogeneity model to a common-homogeneity model it was estimated whether there are different genes that influence the cord characteristics in males and females. Comparing the common-effects gender-limitation model to a homogeneity model it was estimated whether there is any difference in the magnitude of the genetic influences on the aetiology of the cord morphology in males and females.

4.4 Results

Table 4.1 provides the mean cord length and the percentages for the remaining cord characteristics according to twins' gender, zygosity and chorionicity. Cord length, knots and vessels did not differ statistically between DZ (dizygotic) MZDC (monozygotic dichorionic) and MZMC (monozygotic monochorionic) male and female twin pairs. For both male and female twins, cord insertion differed statistically between DZ and MZMC and MZDC twins ($p<0.01$) with more central insertions in DZ twins. In males, MZMC had significantly more peripheral insertions than MZDC twins ($p<0.01$) whereas for females the peripheral cord insertion was high in both MZMC and MZDC compared to DZ twins. A recategorization of the cord insertion into velamentous and non velamentous insertions (as described in the Methods section) led to similar results as those described above. Dizygotic (DZ) male and female twins had significantly ($p<0.01$) more non velamentous insertions compared to the other groups. However, with this categorization we found that MZDC male and female twins had more velamentous insertions ($p<0.01$) compared to MZMC twins. In male twins DZ had significantly more counterclockwise twisting than MZDC and MZMC had significantly more counterclockwise twisting than MZDC ($p=0.02$). Among female twins cord twisting did not differ statistically.

The concordance rates for cord length were MZMC=64%, MZDC=56%, DZ=49%; for knots were MZMC=38%, MZDC=31%, DZ=23%; for insertion were MZMC=0, MZDC=20%, DZ=8%; for number of vessels were MZMC=8%, MZDC=24%, DZ=7% and for twisting were MZMC=9%, MZDC=9% and DZ=8%.

The intra-pair correlations for each cord characteristic according to twins' gender, zygosity and chorionicity are presented in Table 4.2. For cord insertion, the lower intra-pair correlation for female DZ twins (r=0.04) compared to male DZ twins (r=0.12) suggest a possible gender effect. The lower intra-pair correlation in DZ females (r=0.16) compared to DZ males (r=0.24) suggest a possible gender effect for cord knots. For the rest of the cord characteristics intra-pair correlations do not suggest any significant differences between the groups regarding chorionicity or gender effect. Based on the finding that there may be a gender effect for cord knots and insertion gender effect models were fitted to the data to further investigate the gender influence on cord knots and cord insertion heritability estimates.

4.4.1 Univariate genetic analyses

Variance estimates of ACE models and sub-models and their 95% confidence intervals (CI) are presented in Table 4.3, in which the best fitting model is highlighted. The analysis for cord length suggested that genetic factors explained 30% (95%CI: 23-37%) of the variance, a common environmental factor explained 32% (95% CI: 26-38%) of the variance and a non-shared environmental factor accounted for 38% (95% CI; 35-40%) of the variance.

For cord knots genetic factors accounted for 27% (95% CI: 26-38%) of the variance, common environmental factors explained 23% of the variance (95% CI: 14-31%) and non-shared environmental factors explained 50% of the variance (95% CI: 46-53%). For cord insertion 24% (95% CI: 18-30%) of the variance was explained by common

environmental factors and non-shared environmental factors explained 76% of the variance (95% CI: 70-82%).

For cord vessels 41% (95% CI: 40-57%) of the variance was explained by common environmental factors and 59% (95% CI: 43-77%) was explained by non-shared environmental factors. For cord twisting, 34% (95% CI: 29-34%) of the variance was explained by common environmental factors and 66% (95% CI: 66-71%) of the variance was explained by non-shared environmental factors.

4.4.2 Gender-effects genetic models

There was no significant difference between the general sex-limitation model and the common-effects gender-limitation for cord insertion ($\Delta\chi^2$=0.49, df=1) or for cord knots ($\Delta\chi^2$=1.24, df=1). Additionally, there was no significant difference between the common-effects sex-limitation model and the homogeneity model ($\Delta\chi^2$ =6.61, df=4) for cord insertion or for cord knots ($\Delta\chi^2$=3.79, df=4).

4.5 Discussion

The present study, which employed a twin research design with a sample of more than 10,000 twins, is the first to examine the relative contributions of genetic and environmental determinants on the characteristics of the umbilical cord, employing a twin research design to do so with. The heritability analyses suggested that genetic and non-shared intrauterine environmental factors play a substantial role in explaining the majority of the variation of the cord characteristics.

4.5.1 Heritability analysis

The length of the cord is thought to reflect fetal movement *in utero*, often resulting in knotting (162). Reduced cord length is associated with constraint *in utero*, which is more likely to evoke intrauterine problems, with the fetus moving randomly within a confined space during pregnancy. Both short and long cords have been associated with hematomas and thrombosis of the cord vessels and the surface of the placenta, which could lead to the damage of the fetal central nervous system and sometimes even fetal death. Illustratively, cords from fetuses with Down's syndrome, which is normally associated with hypotonicity and reduced fetal movement tend to be shorter with a fewer number of coils compared to healthy fetuses (162). Genetic, common and non-shared environmental factors seem to regulate the formulation of cord length and knots. It can be speculated that monozygotic twins are exposed to a more adverse fetal environment due to different placental circumstances, such as the sharing of one chorion and/or amniotic sacs (163) which can regulate the length of the cord and the knotting. A knot can constrict the blood vessels and lead to fetal death. If the knot is loose, the fetal circulation is maintained albeit at a diminished rate. The tightening knot can preclude the circulation between the placenta and fetus and therefore obstruct the circulation of food supply leading to compromised feeding (119). One possible mechanism of the genetic effect on knots and length is that this could reflect the influence of a genetic predisposition on the amount of twin movement, which in turn impacts on these characteristics of the intrauterine environment.

For cord insertion and number of vessels, the heritability analysis suggested that environmental factors, both shared and individually unique environmental factors

explain most of the variance of cord characteristics. The site of the placental implantation is thought to be important; the upper part of the uterus is the most favourable area for placental implantation because it is rich in blood and therefore nutrients and oxygen. The lower part of the uterus, however, is not and therefore more risks are associated with a low implantation (164). However, certain conditions may predispose to a low implantation. Low implantations of placenta have a strong tendency to migrate upwards toward the body and the base of the uterus, which can shift the cord toward a marginal or even velamentous cord insertion (165). It is believed that the occurrence of marginal cords is specific to the vascular supply and peculiarities of an individual's uterus, including uterine scars, infections or prior pregnancy complications with no signs of genetic predispositions.

Broadly, these results suggest that even in that early stage of life and regardless of the shared environment, individual twin members can uniquely experience situations within the same intrauterine environment. However for cord insertion, chorionicity seems to be an important factor affecting the insertion (discussed below).

The results of this study suggest that non genetic factors affect the twisting of the cord, with shared and non-shared environmental factors explaining most of the variation. Previous research has debated over whether twisting is genetically determined, inherent to the cord itself or the result of external/extrinsic forces. Fletcher (166) talked about a mechanical rotation in which the cord twists as a result of fetal movement or rotation in early gestation. On the other hand, alternative theories have suggested that twisting is the result of factors inherent to the cord itself. Malpas and Symonds (25) have suggested

that the helical structure of the cord results from genetic differences in the direction of the fibres in vessel walls and that a reciprocal action between the vessels walls and flow rate of the fetal blood result in the umbilical cord twist. However, in this study the prevalence of cord twisting for MZ twins compared to DZ is not much higher suggesting that there may not be a profound genetic influence on the aetiology of twisting. On the contrary, unique factors not shared between the twin pair influence the development of twists. It has been previously suggested that fetal activity can explain cord twisting. Some suggest an accentuation of the natural narrowing of the Wharton's jelly toward the fetus permits excessive movement, while others conclude that fetal motion and twisting may cause a reduction of the Wharton's jelly (26). If an umbilical cord is twisted, it is more likely to tighten where there is less resistance, such as an area low in Wharton's jelly. A possible explanation for the unique environmental influences on cord twisting may be due to each twin's different experience of circumstances *in utero*, such as an area low in Wharton's jelly resulting in the twin's unique motor activity.

However, the role of the common environmental factors in influencing twisting is not negligible (14% of the cord twisting variation). Often genes express themselves through the environment. The first environment of the twins is the uterus where the genes from the parents and the genes of each twin operate. However, in the intrauterine environment the genetic influence is likely to be shared, which means that it will impact on both twins equally. The intrauterine shared environment may alter the way the twists are demonstrated and formed in the uterus, but the consequences may be unique to individual twin members.

4.5.2 Chorion and gender effects

The comparison between monochorionic and dichorionic twins revealed that cord length, knots and number of vessels were not statistically different between MZMC, MZDC and DZ twins. In MZMC male and female twins a peripheral/marginal cord insertion was significantly more prevalent than MZDC and DZ male and female twins respectively. This finding is consistent with previous research (149) suggesting that marginal cord insertion in twin placentas, and especially monochorionic and dichorionic twins, is higher compared to singleton placentas. Monochorionic twins, who experience higher intrauterine constraint, may have compromised placental development resulting in abnormal cord insertion into the placenta, increasing the risk of cord vessel rupture (165). Sharing a chorion may influence the site of placental insertion and therefore produce competition for resources within the pairs. Peripheral cord insertion has been associated with low birth weight (86) and thus it is reasonable to accept previous findings supporting the notion that significant discordance in fetal growth may result either from interfetal transfusion or placental insuffiency (155). Unfortunately, further analyses using a genetically sensitive approach to examine the effect of the chorionicity type on cord insertion could not be performed due to the negative correlations between MZMC twins compared to MZDC and DZ twins. Additionally, when the analyses were repeated having recategorized cord insertion as velamentous and non velamentous,it was shown that MZDC twins had significantly more velamentous insertions compared to MZMC twins suggesting that the difference between MC and DC twins should be further explored in future studies.

In MZMC male twins, clockwise/right twisting was significantly less prevalent compared to MZDC male twins. For female twins, cord twisting was not statistically significant different between the three groups. Previous studies comparing right and left twisting have shown that right twisting is associated with more adverse obstetric outcomes (160, 161). However, the results of this study showed that right twisting is less common in monochorionic twins compared to the other two groups, suggesting that monochorionic placentas may not necessarily imply a qualitatively different environment for the development of twisting and its impact on later growth (73, 86). It is possible that the development of cord length, number of vessels and knots in monochorionic twins is due to a similar prenatal environment compared to dichorionic twins.

Finally, the results of the gender effect analysis on the aetiology of cord insertion and knots suggested that there are no gender-specific influences. Differences in the magnitude of the genetic influences were measured by comparing models in which variance components were separately estimated for men and women to models in which they were equated across genders. This analysis suggested that there is no difference between the two models and therefore in the magnitude of the gene expression in males and females.

In conclusion, no previous study has demonstrated the genetic and environmental effects on the variations of umbilical cord pathology and morphology. This study reports that partly genetic and unique environmental factors influence a number of the morphological characteristics of the overall umbilical cord development. Twins, and the

genetic influences on them, can affect their own intrauterine environment, and even in the very early stages of life, twins can experience unique environmental influences.

Table 4.1 Means (SD)/Frequencies of cord morphology by zygosity/chorionicity and gender. ANOVA/Chi2 of cord features: difference between DZ, MZDC, MZMC

	DZ	MZDC	MZMC	P	ANOVA
		Length (cm)/SD			
Male	35.31(11.46)	35.49(11.38)	35.07(10.85)	0.74	-
n	3,041	528	1,209		
Female	33.85(11.13)	33.65(10.24)	33.87(11.11)	0.92	-
n	2,384	537	1,260		
		Knots %			
		Real /False / No			
Male	0.52 / 22 / 77.47	0 /21.89 / 78.11	0.08/ 20.79/ 79.13	0.10	-
n	3,063	530	1,222		
Female	0.31/ 20.32/ 79.39	0.56/22.45 / 76.9	0.24/ 21.17/ 78.59	0.61	-
n	2,869	539	1,266		
		Insertion %			**Chi2**
		Central / Peripheral			
Male	90.71 / 9.29	77.43 / 22.57	71.26 / 28.74	<0.01	DZ>MZMC, MZDC; MZMC>MZDC
n	3,217	545	1,256		
Female	90.48 /9.52	70.65 / 29.35	70.07 / 29.93	<0.01	DZ>MZMC, MZDC
n	3,035	552	1,293		
		Vessels %			
		Two/ Three			
Male	1.13/ 98.97	1.69/ 98.31	1.70/ 98.30	0.21	-
n	3,732	533	1,232		
Female	0.96/ 99.04	1.47/98.53	1.73/ 98.27	0.12	-
n	3,524	545	1,271		
		Twisting %			
		Clockwise/ Mixed or Undefined/Counterclockwise			
Male	61/ 26.41/12.6	66.42/21.32/12.26	59.18/ 26.91/ 13.91	0.02	DZ>MZDC, MZMC>MZDC
n	3,033	530	1,215		
Female	62.35/24.35/ 13.3	62.22 /24.44/ 13.33	60.47/ 25.58/13.95	0.52	-
n	2,834	540	1,247		

DZ, Dizygotic; MZDC, Monozygotic Dichorionic; MZMC, Monozygotic Monochorionic; SD, standard deviation; ANOVA, analysis of variance; n, number of participants

Table 4. 2 Intra-pair twin correlations for each gender by chorion type and zygosity

	Male			Female			OS
Cord morphology	**MZMC (n=640)**	**MZDC (n=276)**	**DZ (n=2,871)**	**MZMC (n=657)**	**MZDC (n=282)**	**DZ (n=2,765)**	**(n=1,882)**
Length	0.62	0.56	0.50	0.67	0.58	0.52	0.49
Knots	0.34	0.34	0.24	0.42	0.27	0.16	0.26
Insertion	0.02	0.25	0.12	-0.03	0.16	0.04	0.08
Number of vessels	0.20	-0.01	-0.01	-0.02	0.57	0.10	0.09
Twisting	0.14	0.08	0.15	0.08	0.20	0.11	0.06

n=number; MZMC, Monozygotic Monochorionic; MZDC, Monozygotic Dichorionic; DZ, Dizygotic; OS, Opposite Sex

Table 4.3 Univariate genetic model-fitting for umbilical cord presenting full and nested models

Threshold of cord morphology	A (95%CI)	C (95%CI)	E (95%CI)	Δx^2	d.f	P	AIC
Length							
ACE *	0.30 (0.23-0.37)	0.32(0.26-0.38)	0.38(0.35-0.40)	-	-	-	-
AE	0.66(0.64-0.68)	(0)	0.34(0.32-0.36)	101.52	1	0.00	99.52
CE	(0)	0.52(0.50-0.55)	0.47(0.45-0.50)	61.46	1	0.00	59.46
E	(0)	(0)	1.00(1.00-1.00)	1526.59	2	0.00	1522.59
Knots							
ACE *	0.27 (0.26-0.38)	0.23 (0.14-0.31)	0.50 (0.46-0.53)	-	-	-	-
AE	0.53(0.50-0.56)	(0)	0.47(0.43-0.50)	22.53	1	0.00	20.52
CE	(0)	0.42(0.39-0.45)	0.58(0.55-0.61)	22.67	1	0.00	20.67
E	(0)	(0)	1.00(1.00-1.00)	463.62	2	0.00	459.62
Insertion							
ACE	0.00(0.00-0.06)	0.24(0.18-0.30)	0.76(0.70-0.82)	-	-	-	-
AE	0.23(0.22-0.29)	(0)	0.77(0.70-0.84)	17.21	1	0.00	15.20
CE*	(0)	0.24 (0.18-0.30)	0.76 (0.70-0.82)	0.00	1	-	-2.00
E	(0)	(0)	1.00(1.00-1.00)	0.00	2	0.00	59.11
Number of vessels							
ACE	0.11(0.00-0.64)	0.33(0.00-0.57)	0.56(0.35-0.77)	-	-	-	-
AE	0.49(0.27-0.67)	(0)	0.51(0.33-0.73)	1.22	1	0.27	-0.78
CE *	(0)	0.41(0.40-0.57)	0.59(0.43-0.77)	0.11	1	0.74	-1.89
E	(0)	(0)	1.00(1.00-1.00)	18.25	2	0.00	14.25
Twisting							
ACE	0.00(0.00-0.06)	0.34(0.32-0.37)	0.66(0.66-0.71)	-	-	-	-
AE	0.39(0.38-0.44)	(0)	0.61(0.56-0.67)	21.78	1	0.00	19.78
CE *	(0)	0.34(0.29-0.34)	0.66(0.66-0.71)	0.00	1	-	-2.00
E	(0)	(0)	1.00(1.00-1.00)	102.37	2	0.00	98.37

A, Additive genetic; C, shared environment; E, non-shared environment; CI, confidence interval; $\Delta\chi^2$, Difference Chi-square; d.f, degrees of freedom; AIC, Akaike's Information Criterion

* Best fitting model

THE TWINS AND MULTIPLE BIRTHS ASSOCIATION

HERITABILITY STUDY

CHAPTER 5

METHODS: THE TWINS AND MULTIPLE BIRTHS ASSOCIATION HERITABILITY STUDY (TAMBAHS)

The set up of the Twins and Multiple Births Association Heritability Study was part of my PhD programme. I was involved in all aspects of setting up this project. My work involved: the development of the research protocol and study materials, obtainment of ethical approval, website construction, recruitment, data cleaning, statistical analyses and writing up for scientific publications. Since the start of the data collection in mid July 2008, information on 1,818 twins has been collected. Although TAMBAHS has collected information on a variety of outcomes, my PhD thesis focused specifically on aspects of early temperament and emotional and behavioural disorders with an emphasis on the intrauterine environment and maternal influence. The study was approved by the University of Birmingham Ethical Review Committee in April 2008.

5.1 General Characteristics

The Twins and Multiple Births Association Heritability Study (TAMBAHS) is a UK nationwide volunteer-based online study of twins and their parents which started in mid July 2008 at the University of Birmingham. The study commenced in collaboration with The Twins and Multiple Births Association (TAMBA) which was the main liaison to the twin clubs in the country. TAMBA is a charity set up by parents, whose main purpose is to help and provide support to families with multiples.

By the end of April 2010, 909 eligible families were identified and information on 1,818 twins from birth until five years old was collected. TAMBAHS collected data via validated questionnaires about maternal lifestyle and child development from two age groups, discussed below in more detail.

5.2 Data Collection

An invitation letter to the study was sent to all present (n=6,100), at the time the study started, members with twins from 160 twin clubs around UK registered with TAMBA. All parents that were lapsed or current members of TAMBA, with healthy twins aged 0-5 years and born in the UK, were invited to participate in the study. Because either the mother or the father could reply to the questionnaire, there were a few cases (n=16) where the fathers of the twins reported on maternal characteristics (such as maternal early pregnancy weight which was tested in chapters 7 and 8). To avoid any bias in the results because of paternal misreporting of the actual weight of the mothers, the results of all analyses using the TAMBAHS sample were checked when the paternally provided data were removed. There was no difference and thus the paternal reports remained in the analyses. A further 4,500 lapsed members with twins were also notified about the study by email. Hardcopy updates were circulated from the TAMBA office throughout the year 2008-2009 and the invitation letter was advertised in the TAMBA regular newsletters.

The invitation letter required only one parent (either the mother or the father) from each twin family to visit the study's website and complete the questionnaire about child development along with some maternal characteristics and information about the fathers of the twins.

5.3 Online questionnaire development

The online questionnaire was constructed using the Select Survey.NET software (versions 3.8.2 and 4.017.005). This software provided a flexible programming

environment for the design and format of the questionnaire. Anonymous access to the questionnaire was enabled at entry to the study.

Respondents were able to resume and finish the survey they started at an earlier date and left incomplete and enter only the questions that were not previously answered. The type of the questions asked ranged from open ended to drop down questions where respondents had to choose between two options the one that best suited them, or multiple answers in a matrix format were enabled allowing respondents to choose more than one option where applicable. See Appendices 3 and 4 for the whole questionnaire.

5.4 Website development

The questionnaire was accessed via the study's website (http://www.tambahs.bham.ac.uk/) which was hosted by the server of the University of Birmingham and developed via the University Content Management System (UCMS) facility. The home page of the website provided a brief introduction to the study (see Appendix 1) and prompted the parent to read more details in the information sheet about the study, under the Frequently Asked Questions (FAQs) tab which appeared on the left hand side of the same webpage. The information under the FAQs (see Appendix 2) was presented in an expandable format in three levels of information, with the first level containing brief information, the second level containing more detailed information and the third level was designed to provide more sophisticated information including links to academic articles or other non-lay sources. This way, the information the participants went through and read could be quantified and the time they spent reading the information in each level could be measured. The amount of information participants

read and the time they spent was further investigated in an empirical study described in Chapter 6.

After reading the FAQs the participants were directed to complete the questionnaire based on their twins'age. Two options were available; parents would complete the first questionnaire if their twins were between birth to eighteen months old (0-18 months old) and the second questionnaire if the twins were between eighteen months to five years old (18 months - 5 years old). On the left hand side of the home page the participants could access further information with regards to the research team, and could contact the researchers if they needed more information about the questionnaire or had any other questions.

5.5 Data collection and management

The data collected on the questionnaires and the information from the FAQs were exported in individual excel files and were then transferred in Stata where they were merged, based on an individual response identification number assigned by the software, into one file for the subsequent data cleaning and statistical analysis. The questionnaire for the 0-18 months old twins included 106 questions and for the twins aged 18 months-5 years old contained 157 questions. For the questions on maternal and child anthropometric information, parents could choose to give their answers using empirical (i.e., pounds) or metric system (i.e., kilograms). For the data cleaning all questions were carefully examined in order to identify inconsistencies in answers about information of twins within a family (i.e., different age for twins in the same family) and other entry

errors on maternal and child characteristics. If any information was given on empirical system it was later converted to metric system's measurements to facilitate the statistical analysis. Some descriptive statistics are presented below for the twin age groups separately (0-18 months old twins, thereafter referred to as 1st age group and 1 ½-5 years old twins, thereafter referred to as 2nd age group).

Particularly, the following information was collected for the twins and their mothers:
Maternal anthropometry and other information: date of birth, age, weight in early pregnancy, weight at the time of the completion of the questionnaire, height, gestational age, employment & education status, ethnic group, contact details. None of the personal contact details questions was compulsory.

Child Anthropometry and other information: twins' age, gender, birth weight and height, weight and height at time of completion of the questionnaire.

5.5.1 1st age group

There were 417 respondents identified (the criteria for eligible responses are described below). The data were complete on twins' age and sex information. Information on maternal age was available for 360 (86%) of the observations; gestational age information was given by 412 (99%) of the mothers; early pregnancy weight information was available for 369 (88%) mothers while body mass index was calculated for 366 (88%) mothers; information on educational level was available for 401 (96%) mothers. The mothers of MZ twins were significantly younger ($p<0.01$) and had lower

gestational age ($p<0.01$) compared to mothers of DZ twins. In addition, MZ twins had lower birth weight ($p<0.01$) and height compared to DZ twins ($p<0.01$).

More information is given in Tables 5.1 and 5.2.

Table 5.1 Maternal characteristics according to twins zygosity (1st age group)

	MZ twins		DZ twins		p-values
	Mean (SD)	**n**	**Mean (SD)**	**n**	
Age (years)	33.5 (4.2)	164	34.7 (4.1)	196	<0.01
Gestational age (weeks)	35.7 (2.8)	185	36.4 (2.5)	227	<0.01
Early pregnancy weight (kg)	66.6 (15.4)	164	69.1 (15.4)	205	0.12
Weight at time of survey (kg)	68.5 (15.7)	165	70.9 (15.2)	197	0.13
Height (m/cm)	1.65 (0.1)	173	1.66 (0.1)	219	0.12
BMI (kg)/ (m^2)	24.4 (5.2)	162	24.9 (5.4)	204	0.31
Employment status	**%**	**n**			
Keeping house or raising children full-time	53.0	95	46.9	106	
Looking for work	-	-	0.4	1	
Unemployed or laid off	0.6	1	0.4	1	0.38
Working full-time	19.0	34	19.5	44	
Working part-time	22.4	40	30.1	68	
None of the above	5.0	9	2.7	6	
Education status					
High School diploma or less	18.6	33	21.1	47	
College/professional education	17.4	31	23.3	52	0.21
University education	64.0	114	55.6	124	
Ethnicity					
White	96.1	172	95.6	216	0.80
Non-white	3.9	7	4.4	10	

Table 5.2 Twins characteristics according to zygosity (1st age group)

	MZ twins		DZ twins		
	Mean (SD)	**n**	**Mean (SD)**	**n**	**p-values**
Age (months)	8.38 (4.9)	376	8.91 (4.7)	458	0.11
Birth weight (g)	2,364 (582)	369	2,548 (651)	449	<0.01
Birth height (cm)	46.5 (5.8)	137	49 (5.2)	186	<0.01
Weight at time of survey (kg)	7. 6 (2.5)	333	7.9 (2.3)	407	0.08
Height at time of survey (cm)	67.7 (10.1)	130	69.6 (8.5)	152	0.08
Gender (%)	45.1	376	54.9	458	0.95

5.5.2 2nd age group

There were 492 respondents identified (the criteria for eligibility are described below). The data were complete on twins' age and sex information. Information on maternal age was available for 441 (90%) observations; gestational age information was given by 489 (99%) mothers; early pregnancy weight information was available for 450 (91%) mothers while body mass index was calculated for 457 (93%) mothers. The mothers of MZ twins were significantly younger ($p<0.01$) and had lower gestational age ($p<0.001$) compared to mothers of DZ twins. In addition, MZ twins were younger ($p<0.001$) and had lower birth height compared to DZ twins ($p<0.001$) More information is provided in Tables 5.3 and 5.4.

The rest of the research questionnaires are described below. All questions for the two age groups were similar, whereas different questionnaires were used to tap information on temperament dimensions (for the 1st age group) and emotional behavioural problems (for the 2nd age group).

Table 5.3 Maternal characteristics according to twins zygosity (2nd age group)

	MZ twins		DZ twins		p-values
	Mean (SD)	**N**	**Mean (SD)**	**n**	
Age (years)	35.5 (4.7)	163	36.7 (4.2)	278	<0.01
Gestational age (weeks)	35.4 (2.5)	179	36.2 (2.7)	310	<0.001
Early pregnancy weight (kg)	67.0 (11.5)	164	68.6 (14.6)	286	0.23
Weight at time of survey (kg)	68.6 (13.0)	167	70.1 (15.3)	291	0.29
Height (m/cm)	1.7 (0.1)	176	1.7 (0.1)	304	0.06
BMI (kg)/ (m^2)	24.5 (4.3)	163	24.8 (5.1)	284	0.60
Employment status	**%**	**N**			
Keeping house or raising children full-time	75	41.9	113	36.3	
Looking for work	-	-	-	-	
Unemployed or laid off	-	-	-	-	0.51
Working full-time	29	16.2	49	15.8	
Working part-time	73	40.8	147	47.3	
None of the above	2	1.1	2	0.6	
Education status					
High School diploma or less	42	25.3	53	18.0	
College/professional education	23	13.9	51	17.3	
University education	101	60.8	191	64.7	
Ethnicity					
White	173	97.2	305	98.1	0.53
Non-white	5	2.8	6	1.9	

Table 5.4 Twins characteristics according to zygosity (2nd age group)

	MZ twins		DZ twins		
	Mean (SD)	**n**	**Mean (SD)**	**n**	**p-values**
Age (years)	3.1 (0.9)	360	2.9 (0.9)	623	<0.001
Birth weight (g)	2,327 (575.8)	354	2,486 (577.8)	609	<0.001
Birth height (cm)	46.5 (5.9)	126	47.3 (7.1)	212	0.27
Weight at time of survey (kg)	10.6 (6.9)	358	10.4 (6.6)	624	0.66
Height at time of survey (cm)	96.0 (0.1)	197	93.9 (0.10)	322	0.02
Gender (%)	36.6	360	63.4	624	0.02

5.6 Zygosity determination

For the determination of the twins' zygosity the adapted version of Goldsmith's zygosity questionnaire was used (167). See Appendix 3. This questionnaire method of assigning zygosity has been validated against determination by identity of polymorphic DNA markers and has reached accuracy in verifying zygosity in 95% of the cases (167). A few individual items of the questionnaire were used as definite markers of zygosity. The twins that were described from their parent as 'alike as two peas in a pod' were classified as monozygotic. This item alone has been shown to correctly classify a high proportion of twin pairs (168). Twins described as 'not looking much alike at all' or as having clear differences in eye colour, hair colour or hair texture were classified as dizygotic, except if they were described previously as alike 'as two peas in a pod' in which case they were not classified and recorded as missing. In all other cases, the items were scored numerically, where low scores were given to responses indicating dissimilar twins. These scores were then summed up and then divided by a maximum possible score on those items that were answered in order to create a physical similarity quotient (PSQ) between 0, which represented maximum physical similarity and 1, representing maximum physical dissimilarity. If the maximum possible score of the questions answered was equivalent to missing data on half or more of the questions the twins were not assigned zygosity.

Since the completion of the zygosity questionnaire preceded the completion of the behaviour and temperament assessment of the twins from their mothers the potential effects of this on the parental responses is discussed below. It is not uncommon that parents of MZ twins may treat their twin offspring more similarly than do parents of DZ

twins (169). If that happens, and if the type of parental treatment in childhood for which MZ twins are more highly correlated than DZ twins significantly influences the risk for adult psychological disorders, then differential treatment of MZ and DZ twins would violate the Equal Environment Assumption (EEA) for psychological/psychiatric twin studies. In order to evaluate empirically the problem of differential treatment as a potential bias in twin studies Kendler and colleagues (170) studied a population-based sample of female-female twin pairs. First, they examined the relationship between perceived parental zygosity and twin resemblance for four major and common psychiatric disorders: depression, generalized anxiety disorders, phobia and alcoholism. Secondly, they examined whether the parental approach to rearing twins differed in parents of MZ versus DZ twins and if so, whether this approach relates systematically to twin similarity for the major disorder. The results showed that mothers' and fathers' beliefs about their twins' zygosity disagreed with assigned zygosity in approximately 20% of the cases. By structural equation model-fitting -where the common environmental path that reflected the effect of parents' perceived zygosity was set to zero- there was no evidence suggesting that mother's or father's perceived zygosity influenced twin resemblance for any of the disorders. Compared to parents of DZ twins, parents of MZ twins were more likely to report that, in rearing their twins, they emphasized their similarities more than their differences. Although these results may suggest there is some evidence for questioning the twin method, it is apparent that differential parental treatment of MZ and DZ twins would invalidate twin studies of psychological/psychiatric disorders only if the type of parental treatment for which MZ twins were more similarly exposed than DZ twins influenced the risk for the disorder under examination. However, when testing the above hypothesis by model fitting, the

parents' approach to raising twins had no significant influence on twin resemblance for the examined disorders. These results suggest that the differential treatment of MZ and DZ twins is unlikely to represent a significant bias in twin studies of these disorders and consequently child development.

5.6.1 Zygosity determination (1st age group)

From all the initial responses (n=700; 1,400 twins) to the questionnaire, 283 responses (566 twins) had no or missing information on more than 50% of the questions or did not have adequate information to allow zygosity classification. From these, although 116 twins (58 twin pairs) were given a physical similarity quotient it was not adequate to assign zygosity reliably (167). Twins not classified as monozygotic or dizygotic and their mothers were not included in the analyses. In total, 417 (834 twins with assigned zygosity) -60% of initial sample-eligible responses were included in the analyses. That means that 86% of the twins who had information to allow the assignment of zygosity were classified as monozygotic or dizygotic.

5.6.2 Zygosity determination (2nd age group)

From the initial responses (n=878; 1,756 twins) in the questionnaire, 386 responses (772 twins) had given no information or information was missing on more than 50% of the questions. This meant that these responses did not provide adequate information to allow the assignment of zygosity. From these, even though 36 twins (18 pairs) were given a physical similarity quotient it was judged as not adequate to assign zygosity reliably (171). Twins not given a zygosity score and their mothers were not included in the analyses. This elimination resulted in 492 (984 twins) respondents included in the

analysis. However, due to further exemption of observations for the purposes of the statistical analyses and based on the low number of mothers being obese, the final sample consisted of 443 observations (886 twins with assigned zygosity). Therefore, 96% of the twins who had information to allow the assignment of zygosity were classified.

5.7 Smoking questionnaire

Maternal smoking was assessed by asking mothers if they smoked before, during and after pregnancy. If they did smoke, they had to report how many cigarettes they smoked on each time period (see Appendix 3). The four dose-response categories were the following: Non-smokers (0 cigarettes), light smokers (1-10 cigarettes), moderate smokers (11-20 cigarettes) and heavy smokers (over 20 cigarettes). The descriptive results on mothers' smoking are presented below individually for each age group.

5.7.1 1st age group

From 402 mothers who completed the questions about smoking before pregnancy, eighty-four percent (84%) reported that they were not smoking before their pregnancy and 16% (n=63) reported that they were smokers before their pregnancy. From these, 34 (54%) were smoking 1-10 cigarettes, 27 (43%) were smoking 11-20 cigarettes and two (3%) were smoking over 20 cigarettes. Ninety-six percent (96%) out of 370 mothers reported that they were non-smokers during pregnancy, while 4% reported that they were smoking during pregnancy. From these, 11 (85%) and two (15%) mothers were smoking 1-10 cigarettes and 11-20 cigarettes respectively. Ninety-three percent (93%)

out of 387 the mothers reported that they do not smoke and 7% (n=28) that they smoke after pregnancy, with 22 (79%) and six (21%) of mothers smoking between 1-10 and 11-20 cigarettes respectively. For more information see table 5.5.

5.7.2 2nd age group

From the 433 mothers who completed the questionnaire on smoking, 80% reported that they were not smoking before their pregnancy and 20% reported that they were smokers before their pregnancy. From these, 44 (51%), 34 (39%) and eight (10%) mothers were smoking 1-10, 11-20 and over 20 cigarettes respectively before pregnancy.

Ninety-seven percent (97%) out of 404 mothers reported that they were non-smokers during pregnancy, while 3% reported that they were smoking during pregnancy. From these 14 mothers, 11 (79%) were smoking 11-20 cigarettes during pregnancy, while three mothers (21%) were smoking over 20 cigarettes a day. Eighty-nine percent (89%) out of 416 mothers reported that they do not smoke and 11% that they smoke after pregnancy. From these 44 mothers who reported to be smoking after pregnancy, 27 mothers (66%) reported that they smoke 11-20 cigarettes, 13 mothers (32%) that they smoke over 20 cigarettes and one mother (2%) answered that she smokes 1-10 cigarettes after pregnancy.

For more information see table 5.5.

Table 5.5 Mother's smoking and number of cigarettes smoked before, during and after pregnancy (both age groups)

	1ST AGE GROUP			2ND AGE GROUP		
Before pregnancy	**1--10 Cig (n/%)**	**11--20 Cig (n/%)**	**over 20 Cig (n/%)**	**1--10 Cig (n/%)**	**11--20 Cig (n/%)**	**over 20 Cig (n/%)**
Smokers	34 (54)	27 (43)	2 (3)	44 (51)	34 (40)	8 (9)
non-smokers	339 (84)			346 (80)		
During pregnancy						
Smokers	11 (85)	2 (15)	-	-	11 (79)	3 (21)
non-smokers	355 (96)			392 (97)		
After pregnancy						
Smokers	22 (79)	6 (21)	-	16 (37)	27 (61)	1 (2)
non-smokers	357 (93)			370 (89)		

The numbers and percentages (adding in 100%) in the table are presented across the rows

5.8 Physical Activity questionnaire

The mothers were asked to report whether they were exercising before and during pregnancy and the hours they spend per week on walking (including to work, shopping or as a leisure activity) cycling (including to work, shopping or as a leisure activity) and housework (cleaning, childcare). They could also add any other activity they were engaged to that was not already mentioned. The descriptive results on mothers' physical activity are presented below for each age group.

5.8.1 1st age group

Eighty-six percent (86%) out of 398 mothers were exercising before pregnancy and 14% were not exercising before pregnancy. Sixty nine percent (69%) of the mothers were exercising during pregnancy and 31% reported that they were not exercising. Mothers were spending on average seven hours per week on walking before pregnancy and five hours during pregnancy. They also reported to be spending almost no time per week cycling before and during pregnancy. They were spending twelve hours per week on housework before and ten hours per week during pregnancy.

5.8.2 2nd age group

Eighty-seven percent (87%) out of 434 mothers were exercising before pregnancy and 13% were not exercising before pregnancy. Seventy percent (70%) of 430 mothers were exercising during pregnancy and 30% reported that they were not exercising. Similarly to the mothers of the younger twins in the dataset, mothers of the older twins were spending on average seven hours per week on walking before pregnancy and five hours

during pregnancy. They also reported to be spending almost no time per week cycling before and during pregnancy. They were spending twelve hours per week on housework before and eleven hours per week during pregnancy.

5.9 Hand and Foot Preference

5.9.1 Modified Edinburgh Handedness Questionnaire

The handedness questionnaire (172) consisted of four items. The questions pertained to handwriting and one other important activity for men and women (i.e., the hand used to write and to put on mascara for women-this item was not in the standard inventory- and hand used for using the hammer for men, or picking up a toy and hand used for holding a spoon if referring to the hand preference of twins. For each item on the questionnaire, hand preference for the particular activity was recorded as "always right" (+2), "usually right" (+1), "no preference" (0), "usually left" (-1) and "always left" (-2). The values per item were summed, divided by the number of questions and multiplied by 100 to yield the handedness preference index (HPI) ranging from -100 to +100 for the mother, the father and each twin separately. The descriptive results for hand preference are presented below.

1st age group

Seventy five percent (75%) of mothers reported a preference for using the right hand, 1% reported that they have no preference for using any of the hands; six percent (6%) reported to be left-handed. The rest of the mothers (5%) could be categorised as usually showing some preference for the left hand and 14% could be categorised as usually showing some preference for the right hand. Similar were the results for the fathers. The

mothers of thirty nine percent (39%) of first born and nineteen (19%) percent of second born twins reported no preference for their twins' hand use. Five percent (5%) of first born and three percent (3%) of second born twins were reported to be right handed. Forty four percent (44%) of first born twins could be categorised as usually showing some preference for the right hand and 16% of second born twins could be categorised as usually showing some preference for the right hand. Twelve percent (12%) of first born twins could be categorised usually showing some preference for the left hand and 7% of second born twins could be categorised as usually showing some preference for the left hand. For more information see table 5.6.

Table 5.6 Parents and twins hand preference (1st age group)

Hand preference	**Right**	**Left**	**No preference**	**Usually right**	**Usually left**	**Total**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	
Mother	264 (74.8)	21(5.9)	3 (0.9)	49 (13.9)	16 (4.5)	353
Father	269 (76.6)	21 (5.9)	4 (1.1)	38 (10.8)	19 (5.4)	351
Twin 1	16 (4.7)	-	132 (39.0)	150 (44.3)	40 (11.8)	338
Twin 2	9 (2.7)	-	62 (18.5)	55 (16.4)	24 (7.2)	150

The numbers and percentages (adding in 100%) in the table are presented across the rows

2nd age group

Eighty percent (80%) of the mothers reported to use the right hand, while 2% reported to be left-handed; two percent (2%) showed no preference; around 4% could be categorised as usually showing some preference for the left hand and around 12% could be categorised as usually showing some preference for the right hand. The results were

similar for the fathers. The mothers of eight percent (8%) of first born and 9% of second born twins reported that their twins showed no hand preference. Twenty three percent (23%) of the first born and 26% of second born twins were reported to be right-handed while 4% of the first born twins and 2% of the second born showed a preference for the left hand. Around fifty percent (50%) of first born and second born twins could be categorised as usually showing some preference towards using the right hand and around 17% of first born and 15% of second born twins as usually showing some preference for the left hand. For more information see table 5.7.

Table 5.7 Parents and twins hand preference (2nd age group)

Hand preference	Right	Left	No preference	Usually right	Usually left	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	
Mother	298 (79.9)	8 (2.1)	6 (1.6)	46 (12.3)	15 (4.0)	373
Father	279 (75.2)	23 (6.2)	10 (2.7)	41 (11.1)	18 (4.9)	371
Twin 1	85 (22.5)	15 (3.9)	30 (7.9)	185 (49.1)	62 (16.5)	377
Twin 2	96 (25.5)	7 (1.9)	35 (9.3)	184 (48.8)	55 (14.6)	377

5.9.2 Modified Waterloo Footedness Inventory

This footedness questionnaire (173) consisted of two items of the form 'Is your foot preference for stomping on a bug "always right" (+2), "usually right" (+1), "no preference" (0), "usually left" (-1), "always left" (-2) taken from the the Modified Waterloo Footedness Inventory. Parents were also asked to give a response regarding their twins' preferred foot when kicking a ball. The footedness preference index (FPI) ranged from -100 to +100. The descriptive results for foot preference are presented below separately for each age group.

1st age group

Fifty eight percent (58%) out of 347 mothers and 55% of fathers reported to show a preference for using the right foot. Equally, five percent of mothers and fathers reported to have a preference for the left hand. Six percent (6%) of mothers and 11% of fathers reported no preference. The remaining 25% of mothers and fathers respectively could be categorised as usually showing some preference for the right foot. Around six to seven percent (6-7%) of mothers and fathers could be categorised as usually showing some preference for the left foot.

Mothers reported that around 81% of the first born and second born twins showed no foot preference. Equally five percent of first born and second born twins were reported by mothers to be right footed. Eleven percent (11%) could be categorised as usually showing some preference for the right foot. The remaining three percent (3%) could be categorised as usually showing some preference for the left foot. For more information see table 5.8.

Table 5.8 Parents and twins foot preference (1st age group)

Foot preference	Right	Left	No preference	Usually right	Usually left	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	
Mother	201 (57.9)	16 (4.6)	22 (6.3)	85 (24.5)	23 (6.6)	347
Father	182 (54.7)	14 (4.2)	36 (10.8)	82 (24.6)	19 (5.7)	333
Twin 1	11 (5.2)	-	170 (80.6)	23 (10.9)	7 (3.3)	211
Twin 2	11 (5.2)	-	169 (80.5)	23 (10.9)	7 (3.3)	210

2nd age group

Sixty five (65%) of 370 mothers reported to always use the right foot and 3% reported a preference for the left foot. Eight percent (8%) reported no preference; twenty two (22%) could be categorised as usually showing some preference towards the right foot and the remaining 3% could be categorised as usually showing some preference for the left foot. Similar were the results for the fathers. Around twenty eight percent (28%) of first born and second born twins showed no foot preference. Twenty three percent (23%) of first born and 24% of second born twins showed a preference for the right foot, while 27% showed no preference. Around 38% of first born and 37% of second born twins could be categorised as usually showing preference towards using the right foot and the remaining towards using the left foot. For more information see table 5.9.

Table 5.9 Parents and twins foot preference (2nd age group)

Foot preference	Right	Left	No preference	Usually right	Usually left	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n
Mother	240 (64.5)	10 (2.7)	28 (7.6)	80 (21.6)	12 (3.2)	370
Father	219 (61.2)	12 (3.4)	28 (7.8)	80 (22.3)	19 (5.3)	358
Twin 1	78 (22.0)	11 (3.1)	99 (27.9)	141 (39.8)	25 (7.1)	354
Twin 2	87 (24.7)	20 (5.7)	98 (27.8)	123 (34.9)	24 (6.8)	352

5.10 Infant Behaviour Questionnaire-Revised (0-18 months old)

The infant temperament was assessed using the revised Infant Behaviour Questionnaire (IBQ-R) (174). The parents were asked to report on a 7-point Likert-type scale the relative frequency of occurrence of specified infant reactions in concrete situations during the the past seven days. The scale ranged from one to seven (never, very rarely, less than half the time, about half the time, more than half the time, almost always, always, does not apply). Scale scores represent the mean score of all scale items. The scores were computed by the following method: a numerical score was not given if an item was omitted, or if the parent checked the "does not apply" response. The total was then divided by the number of items receiving a numerical response.

The IBQ-R consists of 14 scales. For the purposes of this study only four dimensions of temperament were used; 1) Activity Level, which consists of items examining the twins' movement of arms and legs, squirming and locomotor activity; 2) Distress to Limitations which consists of items looking into twins' fussing, crying or showing distress while a) in a confining place or position; b) involved in caretaking activities; c) unable to perform a desired action; 3) Low Intensity Pleasure which consists of items looking into the amount of pleasure or enjoyment related to situations involving low stimulus intensity, rate, complexity, novelty, and incongruity; 4) Duration of Orienting which consists of items on the twins' attention to and/or interaction with a single object for extended periods of time. Reliability (alpha>.70), convergent validity and relative stability have been demonstrated for the original IBQ (175-177).

Four hundred (96%) parents filled in the information on Activity level and Distress to limitations items respectively; 367 (88%) filled in the information about Low intensity pleasure and 373 (89%) completed the information on the Duration of orienting items. The questionnaire can be found in Appendix 3. In Chapter 7 the influence of maternal pre-pregnancy weight on infant temperament is discussed.

Parent-report of temperament is a very common practice especially with children that young in age. On the other hand, observational methods have many advantages over methods such as a self-report or parent-report of child behaviour. However, there are also considerable difficulties in using this approach. Observations provide a direct way of assessing behaviour, where behaviour can be defined consistently and reliably (178). There is evidence that observational data involve less systematic bias than do parent-report measures (179). Among the disadvantages of observational research is that correlations between observed and self-reported behaviour are often low (180) and it is rather time- and resource-consuming for both participants and researchers. Another important concern with this type of research is that of participant reactivity, which, if present, raises concerns about the validity of the data. In more detail, reactivity refers to a few different effects, with the most important being that participants who are being observed may change the behaviour that is being observed. For example, an experimental setting (with the observer present or in a laboratory with only the mother and the child present) the behaviour of the child may change and therefore behaviour may not be estimated reliably. A home setting, where the child interacts with the mother in a familiar place, is adequate to give reliable data.

5.11 Child Behaviour Checklist (18 months-5 years old)

The Child Behaviour Checklist for toddlers (CBCL/1 ½-5) (181) was used to obtain standardised parent reports of children's problem behaviours. The CBCL/1½-5 contains ninety-nine problem items split into seven subcategories: emotionally reactive, anxious/ depressed, somatic complaints, withdrawn, sleep problems, attention problems, and aggressive behaviour originally derived by factor analyses. The broadband scale 'Internalising' is the sum score of items in the first four syndrome scales, whereas 'Externalising' is the sum score of attention problems and aggressive behaviour. 'Total problems' is the sum score of all ninety-nine 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 2 months. The checklist was not scored if data were missing for more than eight items of the total ninety-nine items. If one of the total of the problem scales was missing then the total syndrome score was not calculated. If less than 8 items were missing, they were imputed by using the average of each scale. Good reliability (with most test-retest r's being in the .80s and .90s, has been reported for this checklist (181). Three hundred eighty nine (88%) parents filled in the information on Externalization, Internalization and Total scales. The full questionnaire is presented in Appendix 4. In Chapter 8 the influence of maternal pre-pregnancy weight on the children emotional and behavioural problems is discussed.

5.12 Third factors that could influence the observed associations with temperament and behaviour problems

The next two chapters will make use of the data collected via the TAMBAHS project to investigate 1) the association between maternal pre-pregnancy weight and infant temperament as measured with the IBQ-R (please see 5.10 and chapter 7) and 2) the association between maternal pre-pregnancy weight and behaviour development in pre-school children as measured via the CBCL (please see 5.11 and chapter 8). These associations can be influenced by other "third factors" in three basic ways. Pre-pregnancy weight could have a direct causal influence on a "third factor", which in turn determines the subsequent outcome (Intermediate Factor); pre-pregnancy weight could be associated with the "third factor" in the TAMBAHS sample that is also expected to have a causal association with the outcome creating a Confounding situation; or the "third factor" could not have a direct effect on its own but modify the association between pre-pregnancy weight and infant temperament or behaviour (Effect Modification). Below, I will discuss potential "third factors" and in which way I expect these to have an influence on the relationships tested in chapters 7 and 8.

Smoking

Previous literature has suggested an association between smoking during pregnancy and behavioral problems in childhood and later adolescence, including ADHD, aggression; conduct problems and depression (67, 182, 183) and more difficult temperamental characteristics in infancy (184). Weitzman (185) found that women who smoked both during and after pregnancy rated their children as having more behavioural problems. Brook and colleagues (186) found that mothers who smoked during pregnancy were

significantly more likely to have toddlers who exhibited negativity than did mothers who only smoked after delivery. A positive association (but not causation) is to be expected between maternal BMI and smoking (182, 183). Although the association between maternal smoking and outcomes could have been confounded itself, it is possible that smoking acts as a potential confounder in the regression analyses of Chapters 7 and 8. As only very few mums smoked (around 7%) a confounding effect is unlikely. Nevertheless this potential bias has been tested in subsequent analyses. It is unlikely that smoking would act as an intermediate factor as we would not expect a causal relationship between pre-pregnancy weight and smoking behaviour and because the pathway between maternal BMI and child development is likely to be based on the intra-uterine environment (67).

Socioeconomic Status

Research has shown that children coming from families with a low socioeconomic status (SES) show higher rates of overall problem behaviour (187, 188) (ADD ref from ses.pdf). The effects of SES are particularly consistent for disruptive behaviour disorders (189, 190), ADHD (191), and depression (190, 192). However, not much research is conducted looking into the association between SES and infant temperament. Some studies have found no or very little influence of the socioeconomic status on infant temperament (193) while others have shown that SES inequalities in temperament can be present in six month old infants and can partially be explained by family stress and psychological well-being (194). In addition, the link between SES and children's social and emotional well-being is not as consistent as the link with the cognitive attainment. However, there is evidence suggesting that low-SES children

more often manifest symptoms of psychological problems and maladaptive social functioning than children from more affluent environments (195). Although the literature relating SES with child development is inconsistent, the evidence base regarding the association between BMI and SES is strong. The potential confounding impact of SES has therefore been investigated in chapters 7 and 8.

Parenting

Another important factor related to later outcomes of child behaviour outcomes is parenting style or strategies that parents use to manage child misbehaviour. These have been clearly linked to development of child and adolescent behaviour problems. In particular, parents who are harsh or very permissive with their children are more likely to have children who are behaviourally aggressive (196). Unfortunately, we did not have the relevant data to test for the contribution of parenting and stress into later outcomes. Therefore, if parenting style is associated with pre-pregnancy weight, parenting style could be considered as one of the explanatory factors in the associations found. Indeed parenting style is discussed as a potential reason for the observed association between pre-pregnancy weight and aggressive behaviour in the Discussion paragraph of Chapter 8.

CHAPTER 6

AN EMPIRICAL STUDY ON THE PREFERRED SIZE OF THE PARTICIPANT INFORMATION SHEET IN RESEARCH

6.1 Introduction

Gaining consent is a pre-requisite for nearly all health research involving human participants. The main aim of gaining informed consent is to respect and promote participants' autonomy and to protect them from ignorance about potential harm. European Directive regulations (197) stipulate that participants in clinical trials must be adequately informed about the aims, the method, the expected outcomes and the potential risks associated with study participation. It does not, however, elaborate on what 'adequately informed' amounts to in practice. Jefford and Moore (198) suggest that informed consent requires: 1) the provision of unbiased, up-to-date, relevant information on the consequences of choices; and 2) that the potential participant can freely choose between two or more options (as a minimum whether to enter the study or not). However, they also do not specify the level of detail a potential participant needs to make a choice between the options offered. Current National Research Ethics Service (NRES) guidance suggests that, where appropriate, the Participant Information Sheet (PIS) should be divided into two parts. The first part should contain brief and clear information on the essential elements of the specific study, such as, what is the research about and what participants will have to do. The content of this part should be enough for participants to decide whether they wish to participate in the study. A second part should contain more detailed information, such as data confidentiality, that patients may wish to have (199).

Some studies aimed at improving the readability of the information sheet have concluded that understanding might be improved if the form is easy to read (200-202), and emphasize the need for plain, accessible language (203-205), while others suggest

that understanding and even recall of the information might be enhanced if sufficient time is allowed for reading (206, 207). Short consent forms may also be useful (201, 208, 209).

The extent to which current practice ensures that adequate information is given to potential participants is unclear. Ferguson found that most patients who participated in clinical trials did feel adequately informed and that they were capable of understanding most of the information provided (210). Similarly, Olver et al (211) found that out of 100 cancer patients, 68 felt they had been given the right amount of information, 14 felt there was insufficient information, and only five felt they received too much information. No research so far has empirically and systematically determined the actual amount and type of information people wanted in order to make a decision about participation in research - existing literature only reports on whether, having been provided with a fixed and pre-determined amount of information, participants felt informed.

Not everyone is the same, and some people will want more and some less, information than others. One of the advantages of web-based information is that it can use hypertext markup to make the text interactive and therefore enable users to choose what they want to see and access different levels of information according to their interests and needs. In this study, the participant information sheet was presented in a structured format so that people could get the amount of information they felt they needed. This method of presenting information was safe because this project posed little or no risk to the participants; no clinical interventions or tests were involved and participants were asked

to complete a questionnaire, which could readily be discontinued at any point and the information already recorded discarded. Thus, the risk that participants might be harmed by entering a study on the basis of too little information was negligible.

This study explored how people used the information provided in order to inform future development of information sheets according to participants' actual needs.

6.2 Methods

This study investigates what information people sought or wanted in order to be able to decide whether to participate in the study. The research was embedded as a subproject within the main twin study. The final sample for this study (n=552) consisted of parents who completed the twin survey, and therefore had access to the participant information sheet, between July 2008 and November 2009. Each participant's computer Internet Protocol (IP) was used to track which information was accessed and for how long. The available demographic information enabled the comparison of the information potential participants actually accessed before deciding to participate, by various characteristics (see below).

Before completing the main survey participants were directed to a Participant Information Sheet (PIS) – or as was called in the website Frequently Asked Questions- which offered access to six domains of information in three levels of detail. The domains provided answers to the following questions, which at the time of the study's design were recommended by the UK National Research Ethics Service (199): 1) What is our research about?; 2) Why are we doing this study?; 3) Why have you been invited

to take part?; 4) What would we like you to do?; 5) Who will see the information that is collected?; 6) What will happen to the information that is collected?

To access the information the participant had to click a (+) sign option next to each question. The first level of information was sufficient to give them a broad understanding of the nature of the project and what would be required of them if they chose to participate. The remaining levels were accessed by a deliberate decision of the potential participant by clicking on a second and then a third (+) sign option. The second level was longer and more detailed than the first and provided the reader with what was estimated to be the level of detail required in a standard NRES PIS. The third level was even more sophisticated and normally included links to academic articles or other non-lay sources directly related to the study, containing more information than on a standard PIS. All questions are presented in Appendix 2.

The readability of the PIS in each level was calculated using the Flesch-Kincaid Reading Ease score and grade level. The higher the reading ease score and the lower the grade level the easier it is to read and understand a document. Whether existing readability measurements can accurately evaluate the readability of provided health information is debatable (212, 213). Evidence suggests that the Flesch-Kincaid scale is widely used in studies of readability, has excellent repeatability, and high correlation with other established readability scales (r=0.87 to 0.90) (214) (215). Additionally Kim and colleagues (216) showed that readability scores between four different measurement scales, including the Flesch-Kincaid scale, were similar when compared a health

specific readability measure which takes into account the text unit length alongside semantic and syntactic features of the text.

The information provided in all levels of the PIS had a mean reading ease score of 65.4 and a mean grade level score of 8.8, which indicates that the text was expected to be understood by an average student in the 8th grade (usually around ages 13-14 according to the English educational system) (217). The readability statistics are displayed in Table 6.1. The average time needed to read each domain of the information was calculated based on 200 words per minute, which is the number of words an average person can read in a minute (218, 219).

At the end of the questionnaire, participants could opt to complete a further, short questionnaire about the information they read in the PIS. Participants were asked to choose all options that applied to them from the following list: 1) I didn't click any of the (+) signs options; 2) I didn't find the information under (+) very useful; 3) I didn't find the information under (+) very interesting; 4) I found the information under (+) interesting but it didn't influence my decision to complete the questionnaire; 5) I would not have completed the questionnaire without being able to read the information under (+); 6) I would have liked more information about the project; 7) I would have liked more information about the questionnaire; 8) I would have liked more information about what you are going to do with the results of your study.

6.3 Statistical analysis

Baseline characteristics of the population were recorded. The number of the participants who entered each level of the domains, the actual time they spent reading this information and the number of the participants who assessed the PIS were calculated. To explore whether there were any differences based on the participants' sex, socio-economic status, ethnicity, age or the age of the twins and the information they accessed, the expected mean scores of the maximum level of information accessed and the time spent for every question by each category of the sample characteristics were calculated. All analyses were performed using the statistical software package STATA 11 (220).

6.4 Results

Of those who completed the survey and spent time reading the PIS, 98% (n=540) were women and 2% (n=12) men. With regards to the educational level, 66% (n=309) of the participants had a university education, 20% (n=93) had a college/professional qualification and 14% (n=64) had high school or lower education. Of those who participated, 98% (n=488) were white and 2% (n=10) of other ethnic background (Asian, Black, or Mixed Ethnicity). At the time of the survey, 55% of the participants (n=270) were employed and 45% (n=222) were not employed. With regards to the age of the sample, 8% (n=39) was between the ages of 20-30 years old, 77% (n=360) was between 31-40 years old and 15% (n=68) was between the ages of 41-50 years old (Table 6.2).

Most participants (77%) chose to access the first level of information of each domain. Only 12% accessed the first and second level and 6% accessed all three levels of the domains. More specifically, 82% of the participants accessed the first level of the

question on what participants will have to do, whereas only 11% accessed the first two levels and 7% accessed all three levels of the same question. The first level of the information on what the research was about was accessed by 80% of the participants, while 18% of the participants accessed the first two levels and 12% accessed all three levels. The rest of the questions follow the same pattern with the first level being the more accessed (from 70 up to 76% of the participants) and the remaining of the levels accessed by only a minority (from 3 up to 11% of the participants) (Table 6.3).

The actual time participants spent on each level of every domain is displayed on Table 6.4. The estimated time needed to read the content is also presented. Generally, participants spent more time on the second and third levels of information. On average, the participants spent more time on information about why the survey was being done (25 sec), on what participants were being asked to do (20 sec) on what the research was about (17 sec).

Participants spent around three seconds less than the average reading time on the first level of information about "what would we like participants to do", which was the most accessed information. They also spent less than average reading time for levels two and three of this question. For the second more accessed information relating to "what our research is about", the anticipated reading time was 6.3 sec, whereas the participants spent 7.6 sec (difference of 1.3 seconds) more time reading the first level than the average reading time. They spent more time reading levels two and three but still less time than the average person would need to read and comprehend the content. By contrast, participants spent more than the average reading time on the information

provided on the first level about "why are we doing this research" (difference of 9 seconds).

There was no statistical difference in the pattern of accessing and time spent on the three levels of information between white and non-white ethic groups, or a difference in the educational level of the parents and the age of the twins. Participants aged 41-50 years old spent more time ($p=0.03$) reading the question on 'what is our research about?' than those in the other two age groups. Women were more likely than men to access at least the first level of information concerning 'what is our research about?' ($p<0.01$) and 'why are we doing this research?' ($p=0.02$). Men were more likely than women to spend more time on the information on 'why have you been invited to take part?' ($p<0.001$).

This study also assessed whether participants' perceptions about the quality of the information they read correlated with the actual time spent reading the information provided. The results on how participants perceived the information they read suggested that 34% (n=160) found the information interesting but it didn't influence their decision to complete the questionnaire. Twenty percent of the participants (n=93) would have liked more information about what we are going to do with the results of the study, even though only 6% clicked through to the third level of information. Seventeen percent (n=82) said that they would not have completed the questionnaire without being able to read the information under the FAQs, 15% (n=71) would have liked more information about the project, which again contrasts with the number who actually accessed higher levels of information (see Table 3). Six percent (n=30) would have liked more

information about the questionnaire. Four percent (n=20) said that they did not click any of the (+) sign options (which contrasts with the 18%+ who did not click on any), whilst 3% (n=16) did not find the information interesting and one percent (n=3) did not find the information very useful.

6.5 Discussion

To the best of my knowledge, this is the first empirical study to assess in detail the amount and type of information potential research participants use before they decide to participate in a research study. It recorded how much information was accessed and the actual time spent reading it was compared to the average reading times for the same text. Level 1 information was the most visited while information on levels two and three received much less attention. Few participants accessed level two and three of information and spent little time looking at it, suggesting that the level of detail on standard participant information sheets is not required by most participants in an online survey.

In the case of the most accessed domains in the PIS (information on what the research was about, what participants would have to do and why the survey was being done), the actual time reading and the average reading time for the first and second level were similar, suggesting that participants did read all the information accessed. When accessing the third level of the same domains, however, participants on average only spent approximately half (for the domains on what is our research about and why are we doing this survey) and approximately one third (for the domain on what would we like participants to do) of the average reading time. This may have been because, having

seen what was included they found they were not interested in reading more detailed information; alternatively the level may have been accessed out of curiosity as to what lays behind the 'fold'.

Even though 20% of participants said that they would have liked more information about the study, only 6% accessed the third level of information and only 17% read the information that might be reproduced on a standard PIS (level 2). In short, even when there was information available it was not always utilized. Moreover, participants did not accurately report the extent to which they had actually accessed the information provided. More striking, perhaps, is the proportion of participants who were willing to take part without accessing any information in one or more domains prior to looking at the questionnaire. As Table 3 indicates, between 28 – 30% (depending on the domain) chose not to access *any* information, and between 88-91% chose not to access information comparable to that provided in a standard PIS (level two). When asked about the information provided, 34% stated that reading it did not influence their decision to complete the survey. It can be speculated that rather than relying on the information provided, they went straight to the questionnaire and then decided on the basis of the kinds of questions being asked and the extent to which they found these intrusive, or on whether they felt that their answers would reveal anything they regarded as private or sensitive. It was not possible to tell how many people chose not to participate after looking at either some of the information or the questionnaire itself as information was only collected from those who chose to participate.

Nonetheless, the proportion of those who chose not to access information, or for whom it is reported not to have influenced decision-making cannot be ignored for several reasons. First, it suggests that a significant minority of people did not want or use the information provided when they were actually making a decision about participation in the parent study. This requires further investigation, for example, to determine whether, taken together with the low uptake of information beyond level one, too much weight is being placed on detailed PIS being available to questionnaire studies more generally. Second, taken together with the results on reported use of information and the mismatch between information accessed and the reported need for more information, these results suggest at least a significant minority of participants actually rely less on the PIS to make a decision. Third, the results highlight an ethical question about the responsibilities of researchers using on-line surveys. Programming the on-line system so that potential participants would be unable to sign-up to participate until they had spent at least the average reading time on all domains under level two (which was regarded as being the standard PIS) would not have guaranteed that the information had been read, but it may be regarded as a safe-guard for on-line studies in general. This however, may pose many questions about what constitutes autonomous decision-making. The view that individuals can autonomously chose not to receive 'standard' information when making decisions is not without support, even in conservative bioethics [23]. Unfolding or otherwise interactive electronic information sheets undoubtedly permit potential participants to choose for themselves what information they need to make a decision. Refining them as a means of providing information will mean using them on studies where the risks may be more significant. Taking seriously the idea that information needs vary from person to person means taking seriously the idea that some individuals

may want to know less, and that the duty to inform might be discharged by making a variety of information available rather than by insisting that everyone reads (or at least appears to have read) a fixed amount of information, with the tailoring only coming into play for those whose informational needs exceed this prescribed minimum. The extent to which research ethics committees will be comfortable embracing this as a principle in either research into participants' actual information needs or when applied the more general use of tailored information (where participants can actively chose to know less than may currently be required on a standard PIS) remains to be seen.

One challenge of this study was to determine whether the participants were actually using the information they accessed to inform their decision, given that they could click into a domain and have the browser open without actually reading the material provided. In the case of the first level of information, the comparison with average reading times is strongly suggestive that the text was being read. In the case of the two further levels, things are less clear. The time spent on these domains was generally less than average reading times suggested were required to read them properly. On the other hand, potential participants may skim read through the additional information, either picking out specific sentences of interest or to make sure that there was nothing further that concerned them. Accordingly, they may both value access to the information and consider that it does not influence their decision. Furthermore, the participants to this study knew that they could read through the questionnaire and then decide not to continue – which is easier in the case of internet-based studies than, for instance, personally administered paper questionnaires where it might be harder to decide not to continue when the researcher is present. Internet studies are, however, similar in this

regard to postal surveys, where again, the paper version can be scanned before making a decision about whether or not to complete it.

There are limitations to the generalisability of the results of this study. The participants were predominantly female (98%), white (98%), well educated (66% were university educated and 20% had college or professional qualification) and all were under 50 years of age. The main study was an on-line only study so the participants probably all had reasonably good computer skills and access to the internet. It was not possible to record how many people decided not to participate and therefore, what information was accessed in order to make this decision.

Conclusion

The aim of this study was to examine the amount of information potential participants to the parent study read before they decided to participate, in order to inform discussions about how much information should be contained in a standard PIS. Uisng an innovative way it was measured what information the participants thought they would find most useful and then how long they spent in each information domain. This time spent was then compared to average reading times to determine the likelihood that the participants had actually read all of the information on that domain, identifying what information was most significant for them to read, based on how long they spent reading it. Level 1 information was the most accessed – i.e. the briefest information, which was less than what was being required for a standard PIS. Time spent on these areas was similar to the average reading times suggesting that the information was actually read.

The results on the participants' pattern of accessing and reading information suggested that the majority of the potential participants sought very little information before making a decision about whether or not to participate in this low risk, on-line, questionnaire-based study, and a significant minority felt they needed no information at all.

The NRES guidance for researchers and reviewers (199) has raised the concern that information sheets are becoming increasingly lengthy and complex, and may be deterring participation in clinical research. There is little evidence from which to determine how much information sheet participants actually need. A balance needs to be struck between overwhelming potential participants with too much information and giving them insufficient information to make an informed choice. This study design offered a real possibility for personally tailored information, which may address this concern and improve participant understanding.

Table 6.1 Readability statistics for all levels of domains

PIS	Levels	Number of words	Flesch-Kincaid Reading Ease score (grade level)
1.What is our research about	1	21	72.7 (8.3)
	2	84	54.5 (10.8)
	3	208 plus external link	34.9 (14.1)*
2.Why are we doing this research	1	28	42.4 (14.2)
	2	137	38.8 (14.6)
	3	328	42.2 (13.1)
3.Why have you been invited to take part	1	32	87.7 (5.2)
	2	160	54.4 (12.20
	3	16 plus external link	- **
4.What would we like you to do	1	57	73.2 (7.7)
	2	82	75.3 (6.1)
	3	367	72.4 (8.2)
5.Who will see the information collected	1	30	56.2 (9.1)
	2	257	55.7 (10.8)
	3	150 plus two external links	63.9 (9.1)
6.What will happen to the information collected	1	43	60.4 (8.3)
	2	132	55.0 (11.0)
	3	13 plus external link	27.4 (14.6)

* Readability statistics is derived only from the text provided and not from the content in the links.

* * Could not derive readability statistics on this level

Table 6.2 Frequency distribution table of the main sample characteristics

Participants characteristics	**N=552**	
	n	**%**
Gender		
Female	540	98
Male	12	2.0
Education		
High School Diploma or less	64	13.7
College/Professional	93	20
University	309	66.3
Employment		
Employed/Working	270	54.9
Unemployed/Not working	222	45.1
Ethnicity		
White	488	98
Other	10	2.0
Age		
20-30	39	8.3
31-40	360	77.1
41-50	68	14.6

*n may be less than N in any sub-tabulation due to missing data

Table 6.3 Number/percentage of people who entered/clicked each level for every question

PIS Total n=552	Level 1 n (%)	Levels 1-2 n (%)	Levels 1-3 n (%)	Mean of Levels 1-3 Mean (SD)
1. What is our research about?	446 (80)	78 (18)	53 (12)	1.29 (0.67)
2. Why are we doing this research?	425 (76)	50 (12)	27 (6)	1.18 (0.52)
3. Why have you been invited to take part?	419 (75)	41 (10)	23 (5)	1.15 (0.49)
4. What would we like you to do?	462 (82)	53 (11)	32 (7)	1.18 (0.54)
5. Who will see the information collected?	427 (76)	43 (10)	12 (3)	1.13 (0.41)
6. What will happen to the information collected?	390 (70)	35 (9)	22 (6)	1.14 (0.49)

Table 6.4 Average stay time measured in seconds for each level and estimation of the average reading time needed per level per question (based on average adult reading 200 words per minute)

	Average time spent level1	Average adult reading time level1	**Average time spent level2**	Average adult reading time level2	**Average time spent level3**	Average adult reading time level3	Total time spent
	Mean (SD)		Mean (SD)		Mean (SD)		Mean (SD)
1. What is our research about?	7.6 (7.9)	6.3	24.0 (43.1)	25.2	43.7 (54.3)	62.4	17.4 (34.6)
2. Why are we doing this research?	17.3 (17.7)	8.4	29.0 (23.2)	41.1	50.7 (30.2)	98.4	24.6 (17.1)
3. Why have you been invited to take part?	7.3 (6.9)	9.6	14.3 (10.3)	48	19.3 (26.8)	External link**	10.0 (11.8)
4. What would we like you to do?	14.8 (74.9)	17.1	21.0 (44.2)	24.6	40.1 (19.9)	110.1	20.0 (77.2)
5. Who will see the information collected?	10.5 (66.5)	9.0	21.9 (21.9)	77.1	15.7 (12.9)	45	13.1 (66.7)
6. What will happen to the information collected?	11.9 (39.7)	12.9	17.0 (20.9)	39.6	16.6 (27.8)	Link to scientific paper**	14.5 (40.8)

*The number of words on each level is displayed in Table 1

** The average adult reading time was calculated only for levels that included text and not links to external information

CHAPTER 7

MATERNAL PRE-PREGNANCY WEIGHT AND INFANT TEMPERAMENT

7.1 Introduction

Temperament refers to the individual differences in emotional, motor, and attentional reactivity measured by latency, intensity, and recovery of response and self-regulation processes such as effortful control which modulates reactivity. Differences in individual infant responses can therefore be assessed behaviourally and understood biologically. Temperament is considered to be present early in life and relatively stable across time (50). Thomas and Chess (221) were among the first to emphasize the importance of temperament in infant behaviour and proposed nine dimensions (i.e, activity level, rhythmicity, adaptability, approach-withdrawal, mood, intensity, attention span-persistence, distractibility, threshold of responsiveness) of temperament based on observations of infants' behavioural responses in daily routine interactions with their mothers. Among the subsequent adaptations of this multidimensional approach of temperament was the model proposed by Rothbart (222) suggesting that infant temperament can be measured by the degree to which infants react and regulate their responses. While reactivity is the response to external stimuli, regulation is the manner in which the infant returns to homeostasis.

In modern temperament research (223, 224) specific traits of temperament are thought to form higher-order dimensions. The first of these higher-order dimensions, known as negative affectivity, refers to the degree of sensitivity to signals of punishment (i.e. distress to limitations) and the tendency of an individual to experience negative emotions. The second, surgency/extraversion, refers to the individual's activity level and their tendency toward sensation seeking and positive emotions. The third, emotional regulation or duration of orienting (alternatively referred to as effortful control),

describes the individual's ability to persist, pay attention and modulate emotional responses in novel settings.

An increasing body of literature has suggested a link between temperament dimensions and child psychopathology (225, 226). Specifically, four dimensions of the broad temperamental components: *activity level* (gross motor activity, including squirming and locomotor activity), *distress to limitations* (fussing, crying or showing distress when confined in a place/position or when unable to perform a desired action), *low intensity pleasure* (amount of enjoyment related to low stimulus intensity, novelty and incongruity) and *duration of orienting* (attention to and/or interaction with an object) have been linked with later development of psychopathology (49, 227, 228).

Infants high in distress are at greater risk of showing externalizing problems such as aggression (229) while high activity level and low attention have been linked with attention problems (43, 230, 231). Certain evidence suggests that distress, usually manifested as excessive crying, in the first year of life is predictive of attention problems at school age (232). In addition, persistent problems with behavioural control, such as the inability to stop crying and to regulate feeding and sleeping behaviour in infancy are precursors of behaviour control difficulties such as hyperactivity or conduct problems in childhood (45, 233, 234). Moreover, difficulties with anger regulation are associated with attention problems from preschool through adulthood (46, 47), while focused attention is influenced by external stimuli like the novelty and the intensity of the stimuli presentation (235).

Previous research has examined the aetiological factors of temperament development and has found that genetic and environmental factors influence dimensions of infant temperament (236-238). Twin studies provide strong evidence of genetic influence on temperament, across a wide variety of temperamental dimensions including among others activity (239), distress (237), attention and negative affect (240-242). Estimates of heritability suggest that genetic factors account for 20% to 60% of the variability of temperament within a population.

Apart from the established genetic basis of temperament, the prenatal environment seems to play an important role on later development of temperament (48, 228). There has there been an interest by researchers in interpreting these disorders within the Developmental Origins of Health and Disease Hypothesis using examples of *in utero* risk factors associated with psychological/psychiatric conditions (243).

Mother's weight before pregnancy has only recently been studied as an in utero risk factor for later developmental abnormalities, and it has been found that maternal pre-pregnancy weight is associated with infants' high degree of distress and inattention (66, 244, 245). It has been hypothesized that maternal adiposity at the time of conception may be important for child mental health programming, because prenatal development of the brain depends on maternal energy supply (246). An association has been found between nutrition and temperament and the subsequent development of later psychopathology. This association has been used to support a theory, which suggests that there is a pathway from nutritional deficiencies to areas of brain development that mediate individual differences in temperament, posing a further risk for the

development of internalizing and externalizing disorders (65). There is evidence to suggest that the optimal fetal brain development depends on the energy and nutrition supplied by the mother (64, 247). However, results from a recent study (67) show no evidence of any association between maternal weight and behavioural problems even after adjusting for environmental confounding.

A better understanding of the factors that affect temperament and the potentially complex relationships between genetic and environmental risks, child temperament, and the development of psychopathological behaviour, is particularly important for developing and defining effective targets for clinical interventions and preventive efforts.

The focus of the present study is on the following temperamental dimensions: activity level, duration of orienting, distress to limitations and low intensity pleasure. These have been previously studied as early precursors of ADHD. While it has been suggested that there is a biological basis of the temperament dimensions this basis is not assumed to be merely genetic (248). Genetic and familial factors may be present causing a confounding to the association between maternal weight and child outcomes.

By employing a twin study design, the genetic and environmental etiology of infant temperament will be investigated, as well as the relationship between maternal pre-pregnancy weight and temperament, assessing the differences or similarities between Monozygotic (MZ) and Dizygotic (DZ) twins.

7.2 Methods

The methods for recruitment have been previously described in Chapter 5. In brief, the Twins and Multiple Births Association Heritability Study (TAMBAHS) is a volunteer-based study investigating the development of twins from the age of birth until 5 years of age. To recruit individuals for the current study, an invitation letter was sent to all present and lapsed registered members with twins of the Twins and Multiple Births Association (TAMBA) in July 2008. This requested one parent (either the mother or the father), from each family, to visit the study's website and complete a questionnaire about infant development along with some maternal characteristics.

Twins aged from birth up to 18 months old at the time of the survey were identified. In the time period between July 2008 and May 2010, 417 parents of eligible twins (98.3% mothers, and 1.7% fathers) completed the online questionnaire on their twins' emotional and behavioural development.

7.2.1 Zygosity determination

For the determination of the twins' zygosity the adapted version of Goldsmith's zygosity questionnaire was used (171) (See Appendix 3). This adapted questionnaire as a method of assigning zygosity has been validated against determination by identity of polymorphic DNA markers and has reached accuracy in verifying zygosity in 95% of cases (167).

7.2.2 Twin Sample

834 twins were included in the analyses; 188 monozygotic (MZ) male twins, 188 monozygotic (MZ) female twins, 120 dizygotic (DZ) male twins, 118 dizygotic (DZ) female twins and 220 opposite-sex twins (106 male-female and 114 female-male twins).

7.2.3 Measures

Maternal pre-pregnancy weight

Pre-pregnancy BMI (kg)/(m^2) was analysed both as a continuous and as a categorical variable. Pre-pregnancy BMI was classified according to the WHO standard guidelines as: underweight (<18.5kg) normal weight (18.5kg-24.99kg), overweight (>=25kg) or obese (>=30kg).

The Infant Behaviour Questionnaire-Revised (IBQ-R)

Infant temperament was assessed using the revised Infant Behaviour Questionnaire (IBQ-R) (174). The parents were asked to report on a 7-point Likert-type scale the relative frequency of occurrence of specified infant reactions in concrete situations during the previous seven days. The scale ranged from 1 to 7 (never, very rarely, less than half the time, about half the time, more than half the time, almost always, always, does not apply). The IBQ-R consists of 14 scales. For the purposes of this study we used only four dimensions of temperament; 1) Activity level, which consists of items examining the twins' movement of arms and legs, squirming, and locomotor activity; 2) Distress to Limitations which consists of items looking into twins' fussing, crying or showing distress while a) in a confining place or position; b) involved in caretaking activities; c) unable to perform a desired action; 3) Low Intensity Pleasure which

consists of items looking into the amount of pleasure or enjoyment related to situations involving low stimulus intensity, rate, complexity, novelty, and incongruity; 4) Duration of Orienting which consists of items on the twins' attention to and/or interaction with a single object for extended periods of time. Reliability, convergent validity, and relative stability have been demonstrated for the original IBQ (175, 176). The internal consistency for the IBQ-R items was high with the Cronbach's alpha ranging from .81 to .90.

7.3 Statistical analysis

7.3.1 Correlations

Intra-pair twin correlations were calculated to examine the genetic and environmental influences on the temperament dimensions. The Pearson's (r) and Spearman rho coefficient statistics were used for continuous and ordinal data as appropriate.

7.3.2 Heritability analyses

Univariate genetic models were fit to the data to estimate the heritability of the temperament scales using a maximum likelihood approach implemented in Mx (249). The estimates of the heritability are presented with 95% Confidence Intervals (CI) and goodness of fit statistics for 3 models: a full ACE model, in which the phenotypic variance is explained by genetic (A), common environmental factors (C) and non-shared (E), environmental factors, a model in which all variance was attributed to genetic and non shared environmental factors (AE), and a model in which all variance was explained by non shared and common environmental factors (CE). Reduced models were estimated by removing one of the parameters at a time and re-running the model.

The goodness of fit of the reduced models was compared to the full model to assess whether they represented a better explanation of the data using the likelihood ratio χ^2 test and the Akaike Information Criterion (AIC). The models were assessed by examining the decrease in the fit of the model; if a parameter could be dropped without a significant decrease in fit then, on the grounds of parsimony, the reduced model was accepted as the best fitting model. A model where maternal BMI was included in the models as a measured parameter was fitted to the data in order to estimate the additional influence of this variable on the temperament scores. The fit of this model can be compared to the model without BMI to examine the significance of pre-pregnancy weight.

7.3.3 Regression analyses

Linear regressions

A regression model was used, where the intercept of each twin pair was modeled as a function of the population intercept plus a unique contribution of the twin pair. The expected scores for each temperament scale and the standard errors for each level/category of maternal weight were estimated. The regression coefficients for maternal weight as a continuous variable are also presented. Gestational age, twins' age, twins' gender, birth weight, maternal smoking (before, during and after pregnancy) and maternal educational level were adjusted in the models.

Logistic regressions

Logistic regression models were used where the intercept of each twin pair was modelled as a function of the population intercept plus the individual contribution of the

twin pair. The primary exposure variable was maternal pre-pregnancy BMI, which was calculated as the weight (kg)/ height (m^2) and was used both as categorical and continuous variable. Associations of maternal pre-pregnancy weight and the four temperament dimensions were explored with the temperament scores divided into scores below the median and scores above the median and analysed using the following statistical models: 1) maternal pre-pregnancy weight models unadjusted and 2) maternal pre-pregnancy weight adjusted for gestational age, twins' age, twins' gender, birth weight, maternal smoking (before, during and after pregnancy) and maternal educational level. Both regression analyses were performed using STATA 11 (220).

7.4 Results

7.4.1 Heritability analysis

The means, standard deviations, and within-pair Pearson correlations for the temperament scales stratified by zygosity are presented in Table 7.1. The intra-pair twin correlations for MZ and DZ twins were calculated. For Activity Level, the MZ correlation was r=0.75 and the DZ correlation was r=0.45. For Distress to Limitations, the MZ and DZ correlations were r=0.83 and r=0.56 respectively. For Duration of Orienting, the MZ and DZ correlations were r=0.94 and r=0.85 respectively and for Low Intensity Pleasure, the MZ correlation was 0.99 and the DZ correlation was 0.99.

Variance estimates of ACE models and sub-models with their 95% Confidence Intervals (CI) are presented in Table 7.2, in which the most parsimonious model is highlighted. For Activity Level, an AE model was the most parsimonious with genetic factors explaining 75% (95 % CI: 69-79%) of the variance and non shared environmental factors explaining 25% (95% CI: 21-31%) of the variance. For the remaining three

dimensions, an ACE model was the most parsimonious model. For Distress to Limitations and ACE model was the most parsimonious with genetic factors explaining 52% (95 % CI: 36-72%) of the variance, common environment explaining 31% (95% CI: 11-46%) of the variance and non-shared environment explaining 17% (95% CI: 14-22%) of the variance. For Duration of Orienting an ACE model was the most parsimonious one with genetic factors explaining 19% (95 % CI: 13-26%) of the variance, common environment explaining 76% (95% CI: 68-82%) of the variance and non-shared environment explaining 5% (95% CI: 4-7%) of the variance. For Low Intensity Pleasure an ACE model was the most parsimonious with genetic factors explaining 0.01% (95 % CI: 0-2%) of the variance, common environment explaining 99% (95% CI: 98-99%) of the variance and non-shared environment explaining almost none of the variance. When BMI was included in the models it did not provide a good fit to the data for all the temperament scores and the best fitting models are presented without the BMI parameter.

Sample characteristics of the mothers and the twins stratified by zygosity are presented in Table 7.3. The mean gestational age ($p<0.001$) and birth weight ($p<0.001$) of the MZ twins were significantly lower compared to DZ twins. The mothers of the DZ twins were significantly older ($p<0.001$) compared to the MZ twins. Mothers BMI mean score was 24.67 (95% CI: 24.29-25.05).

7.4.2 Regression analysis

Linear Regressions

There were no significant relationships observed between maternal pre-pregnancy weight and the four temperament scales in the unadjusted models. However, after adjustment there was a statistically significant decrease (p=.02) observed in the Distress to Limitation score with every unit increase of mother's BMI.

Logistic regressions

There were no significant associations observed between maternal pre-pregnancy weight and the four temperament scales. In unadjusted models as well as in adjusted models no associations were observed. However, overweight and obese mothers were more likely to have a child with a lower score in the distress to limitations scale (OR=.72 and OR=.64 respectively) compared to normal weight mothers. Underweight mothers were also more likely (OR=.38) to have a child with a lower score in the distress to limitations scale.

7.5 Discussion

The aim of this study was to examine the relationship between maternal pre-pregnancy weight and infant temperament, and to estimate the genetic and environmental effect on temperament. The heritability analysis results show that distress to limitations and activity level are highly heritable, with genetic factors accounting for 52% up to 75% of the variability respectively. Previous studies (239) have found that genetic factors explain up to 30% of the variability in activity level, for example, whereas in this

dataset a much greater influence of genetic factors for this aspect of temperament was found. On the other hand, common environment accounted for 76% and 99% of the variation in duration of orienting and low intensity pleasure, both of which are expressions of effortful control. Given that temperament is thought to be biologically based, these results suggest that some aspects of temperament are more genetically influenced than others.

While many have argued that temperament is biologically based (250), Rothbart (222) has argued that "temperamental characteristics can be influenced over time by heredity, maturation and experience". This implies that aspects of temperament can be influenced by shared environmental influences on the children. It has also been suggested that temperament is related to emotional and motivational (incentive-based) aspects of behaviour which are likely to reflect environmental influence. Previous twin studies (251) (238) examining the duration of orienting and low intensity pleasure have also found that temperament has moderate or very low heritability in the neonatal period. This may imply that for these temperament dimensions, incentive-response systems may not yet be employed during these early stimulus-response stages of development.

Previous literature suggests that exposure to adverse intrauterine environment increases the risk of emotional and behavioural problems later in life (66, 252, 253). Our results, contrary to previous findings, suggested that there was a decrease in children's distress to limitations scores with every increase in maternal weight, suggesting a protective effect of maternal overweight on children's difficult temperament. However, these effects are seen for the underweight mothers as well which suggests that the level of maternal energy supply may have an important programming effect on the emotional health of children but the association does not have a clear direction. These results are

rather puzzling and further research is needed in order to disentangle the true association between maternal weight and child emotionality.

One possible explanation for the association between overweight and low distress to limitation scores may be the following: The first environment of the child is the uterus where the parental genes and the genes from the child operate. Maternal weight is a marker of the intrauterine environment that may influence later child development. However, it is difficult to sometimes distinguish between the maternal genes transferred to the child and the genes that may be masked by the social experience or personality of the mother which may also genetically influence the child development. An example of that is the association that has been suggested between neuroticism, high impulsivity and low order and overweight (254). Overweight mothers may have difficulty resisting cravings and lack methodological and organized behaviours. Therefore, it may not be the overweight that affect child temperament but rather the specific personality characteristic of the mother that may be genetically transferred to the child, causing a spurious relationship between maternal overweight and infant temperament. In a similar way, the mother may be more soothing regarding her children's needs which lead to children with less emotional problems.

Although this is a very important factor in the investigation of the association between mother's weight and child characteristics, data on other maternal characteristics were not available to further explore this hypothesis and further research is recommended on this topic.

Evidence from studies examining three large pregnancy cohorts has suggested an association between maternal pre-pregnancy overweight or obesity and symptoms of attention-deficit/hyperactivity disorder (ADHD) in school-aged children (66). This association was also observed in a follow-up study of 5 year old Swedish children, which reported associations between maternal weight and child attention and behavioural problems (245). In an effort to identify whether the intrauterine environment or familial confounding factors operate behind these associations, Brion and her colleagues (67) examined the link between maternal overweight and child behaviour problems. Findings from both British (Avon Longitudinal Study of Parents and Children–ALSPAC) (255) and Dutch (Generation R cohort) (256) cohorts adjusting for familial confounders including paternal BMI ratings and education, attempt to distinguish between the shared familial, genetic, and environmental influences. They found no evidence of any association between maternal pre-pregnancy weight and child behavioural problems or cognitive abilities. Therefore, the evidence on the association between maternal pre-pregnancy weight and child behaviour problems remains inconsistent even though the studies have all been conducted in large cohort prospective and longitudinal studies.

As outlined above, associations between children problems and maternal pre-pregnancy weight have been observed in the past but no study had so far investigated whether maternal pre-pregnancy weight can have an effect on infant temperament. Moreover, the argument that temperament is related to attention problems can be criticized by pointing to the nature of the link between temperament and this psychopathological trait (257). Various suggestions have been put forward for the explanation of the relation between temperament and psychopathology. Of the models that have been suggested,

one poses that temperament can be considered as a spectrum, or a common cause model, with normal and abnormal falling at different points on the same continuum. In essence, this model considers temperament to be a sub-clinical manifestation of psychopathology, with shared etiological determinants. The results of this study suggest that some of the aetiological factors important in emotional/behavioural problems, including maternal pre-pregnancy BMI, are not important for temperament. This suggests that temperament is not simply a manifestation of psychopathology with a shared etiology. Shiner and Caspi (258) have argued that it is implausible that complex behaviour such as child psychopathology is the simple product of one or two temperamental factors. Instead they have argued that these temperamental factors likely interact with each other and with other variables such that the "true" impact of temperament is larger than the effect of the individual temperament factors themselves. Evidence for this type of interaction of temperament with other aetiological factors has been provided by Owens and colleagues (259) who found that high levels of emotionality in children are linked to lower levels of responsiveness in mothers, which, in turn, may compromise the establishment of a secure attachment relationship. The lack of a secure attachment, then, could further enhance the risk of the development of internalizing/externalizing psychopathology (260).

In sum, the results of this study provide evidence that some aspects of infant temperament are heavily influenced by genetic factors (distress to limitations, activity level) whereas others are more influenced by environmental factors (duration of orienting, low intensity pleasure). These findings suggest that temperament is a complex

constellation of characteristics with varying influences, as opposed to a unified, biologically driven system, as has been suggested by some.

Table 7.1 Descriptive statistics of temperament scales for monozygotic and dizygotic twin pairs

	Monozygotic twin pairs (n=188)							Dizygotic twin pairs (n=229)						
	Twin 1			Twin 2				Twin 1			Twin 2			
	N	M	S	N	M	S	r	N	M	S	N	M	S	r
Temperament scales														
Activity level	185	4.12	1.01	185	4.00	.93	.75**	215	4.13	.98	215	3.99	.95	.45**
Distress to Limitations	185	3.48	1.02	185	3.55	.98	.83**	215	3.57	.99	215	3.58	.97	.56**
Duration of Orienting	171	3.72	1.20	171	3.69	1.21	.95**	202	3.57	1.13	202	3.53	1.18	.85**
Low Intensity Pleasure	166	4.88	1.01	166	4.88	1.01	.99**	201	4.69	1.05	201	4.69	1.03	.99**

N; Number of twins
M; Mean
S; Standard deviation
r; Within-twin correlations
Twin 1 is the first born
Twin 2 is the second born

Table 7.2 Univariate genetic model-fitting for temperament scales presenting full and nested models

	A (95%CI)	C (95%CI)	E (95%CI)	Δx^2	P	AIC
Activity Level						
ACE	0.59 (0.37-0.78)	0.15 (0.00-0.35)	0.26 (0.21-0.32)	-	-	-
AE*	0.75 (0.69-0.79)	(0)	0.25 (0.21-0.31)	1.92	0.17	-0.08
CE	(0)	0.58 (0.52-0.65)	0.42 (0.35-0.48)	28.97	0.00	26.97
Distress to Limitations						
ACE*	0.52 (0.36-0.72)	0.31 (0.11-0.46)	0.17 (0.14-0.22)	-	-	-
AE	0.83 (0.79-0.86)	(0)	0.17 (0.14-0.22)	9.01	0.00	7.01
CE	(0)	0.69 (0.63-0.74)	0.31 (0.26-0.37)	41.13	0.00	39.13
Duration of Orienting						
ACE*	0.19 (0.13-0.26)	0.76 (0.68-0.82)	0.05 (0.04-0.07)	-	-	-
AE	0.94 (0.92-0.95)	(0)	0.06 (0.05-0.08)	106.79	0.00	104.79
CE	(0)	0.89 (0.87-0.91)	0.11 (0.09-0.13)	41.15	0.00	39.15
Low Intensity Pleasure						
ACE*	0.01 (0.00-0.02)	0.99 (0.98-0.99)	0.00(0.00-0.01)	-	-	-
AE	0.99 (0.99-1.00)	(0)	0.00 (0.00-0.00)	655.14	0.00	653.14
CE	(0)	0.99 (0.99-1.00)	0.01 (0.00-0.01)	32.19	0.00	30.19

* Best fitting model

A, Additive genetic; C, shared environment; E, non-shared environment; CI, confidence interval; $\Delta\chi^2$, Difference Chi-square; P, statistical significance $p<.05$; AIC, Akaike's Information Criterion

When the A and E parameters were sequentially dropped from the ACE model for the Low Intensity Pleasure scale there was a significant deterioration in fit $p<0.000$. The actual confidence intervals for the A estimate (0.0084) were CI: 0.0053-0.0123 and for the E estimate (0.0029) were CI: 0.0022-0.0038

Table 7.3 Means/frequencies, standard deviations/percentages and Pearson correlations with the four temperament scales for each covariate, stratified by zygosity

	MZ twins						DZ twins					
Maternal Characteristics	**Mean**	**SD**	**r $_a$**	**r $_b$**	**r $_c$**	**r $_d$**	**Mean**	**SD**	**r$_a$**	**r $_b$**	**r $_c$**	**r $_d$**
Pre-pregnancy BMI	24.36	5.15	-.08	-.05	.09	-.02	24.92	5.36	-.05	-.13*	.00	-.08
Age (years)	33.47	4.22	-.12*	-.05	-.10	.04	34.65	4.05	-.19**	-.14***	-.09	-.04
Gestational age (weeks)	35.69	0.14	.04	-.06	.06	.03	36.40	0.12	.03	.03	-.04	-.11*
Smoking (no/yes)	**n**	**%**	**r $_a$**	**r $_b$**	**r $_c$**	**r $_d$**	**n**	**%**	**r$_a$**	**r $_b$**	**r $_c$**	**r $_d$**
Before	150/28	84/16	-0.03	-0.02	0.03	0.00	189/35	84/16	-0.03	0.09	0.04	-0.00
During	160/4	98/2	0.00	0.07	0.03	-0.01	195/11	95/5	0.02	0.07	-0.02	-0.05
After	159/11	94/6	-0.04	0.02	0.16***	-0.01	200/17	92/8	-0.03	0.09	0.9***	-0.05
Educational level												
High School diploma or less	33	18.6					47	21.1				
College/professional education	31	17.4	-0.15	-0.11	-0.07	0.04	52	23.3	-0.06	-0.13	-0.05	0.03
University education	114	64.0					124	55.6				
Twin Characteristics												
Birth weight (in grams)	2363.97	581.71	.08	-.03	.05	.03	2547.62	650.91	-.00	-.03	-.02	-.09
Age (months)	8.38	0.25	.35**	.11*	.10*	-.02	8.91	4.70	.16**	.04	.20**	.08
Sex	**N**	**%**					**N**	**%**				
Male	188	50.00	-.14**	-.16**	-.04	.03	120	26.20				
Female	188	50.00					118	25.77	-.03	-.11*	.07	.07
Opposite sex	-	-					220	48.03				

r $_a$ Pearson/Spearman correlation with activity level
r $_b$ Pearson/Spearman correlation with distress to limitations
r $_c$ Pearson/Spearman correlation with duration of orienting
r $_d$ Pearson/Spearman correlation with low intensity pleasure

*p<.05; **p<0.001; ***p<.01

Table 7.4 Logistic and linear regression for the temperament characteristics of the twins based on maternal BMI (used as continuous and categorical)

			Unadjusted		Expected mean	St Error	P	Adjusted*		Expected mean	St Error	P
Outcomes by BMI	Below median	Above median	OR	95% CI				OR	95% CI			
Activity Level**	N	N										
Normal weight	106	111	Ref		4.06	.06		Ref		4.06	.06	
Underweight	6	7	1.40	.63-3.12	4.02	.05		1.31	.54-3.19	4.01	.05	
Overweight	43	37	.86	.60-1.24	3.97	.06	.29	.74	.49-1.10	3.95	.07	.19
Obese	24	18	.84	.53-1.36	3.93	.09		.88	.49-1.11	3.90	.10	
BMI (continuous)			.97	.91-1.04	-.01^	.001	.19	.98	.91-1.04	-.01^	.01	.13
Distress to Limitations**												
Normal Weight	100	117	Ref		3.61	.06		Ref		3.61		
Underweight	8	5	.54	.24-1.22	3.54	.05		.38**	.15-.94	3.51		
Overweight	44	36	.79	.55-1.13	3.47	.07	.10	.72	.48-1.07	3.41		.03
Obese	22	20	.75	.47-1.20	3.40	.10		.64	.38-1.09	3.31		
BMI (continuous)			.96	.89-1.02	-.02^	.01	.06	.95	.89-1.01	-.02^		.02
Duration of Orienting**												
Normal weight	100	103	Ref		3.55	.08		Ref		3.49	.08	
Underweight	6	6	1.11	.48-2.54	3.56	.06		1.56	.64-3.79	3.53	.06	
Overweight	36	42	1.20	.83-1.74	3.57	.09	.86	1.23	.82-1.84	3.57	.09	.49
Obese	22	14	.79	.50-1.32	3.58	.13		.94	.54-1.64	3.61	.14	
BMI (continuous)			1.05	.88-1.25	.008^	.01	.48	.93	.81-1.06	.01^	.01	.56
Low Intensity Pleasure**												
Normal weight	101	99	Ref		4.78	.07		Ref		4.77	.07	
Underweight	6	6	1.02	.45-2.32	4.74	.06		1.10	.46-2.67	4.74	.06	
Overweight	41	33	.87	.59-1.27	4.70	.08	.43	.87	.58-1.31	4.71	.09	.61
Obese	18	20	1.13	.69-1.85	4.66	.12		.99	.58-1.71	4.68	.13	
BMI (continuous)			1.00	.80-1.25	-.01^	.01	.29	.88	.74-1.03	-.01^	.01	.27

*Adjusted for gestational age, twins' age, sex, birth weight, mother's educational level, smoking (before, during, after pregnancy)

**Categorised based on median (above/below median)
***Median for Activity level=4; Distress to Limitations=3.46; Duration of Orienting=3.6; Low Intensity Pleasure=4.8 N; number of mothers in each category
^ Regression coefficient

CHAPTER 8

MATERNAL PRE-PREGNANCY WEIGHT AND BEHAVIOUR PROBLEMS IN PRESCHOOL CHILDREN

8.1 Introduction

A child's preschool years are generally considered important since during this period the critical emergence of many clinically significant problem behaviours occur (40). Externalizing (e.g., aggression, conduct problems, hyperactivity) and internalizing problems (e.g., emotional problems, anxiety, depression) can often first be identified in early childhood which then show considerable stability across older ages (261-263). Toddlers with problem behaviours are at risk for a variety of adverse developmental outcomes including conflictual relationships with other peers or family, poor academic performance, delinquency and later maladjustment (264-266). Given the life course implications of early onset symptomatology, it is essential to understand the underlying aetiology of problem behaviours in preschool children.

Twin studies investigating externalizing and internalizing problems have revealed substantial genetic influence, with heritabilities ranging from 40-70% in these age groups (267-269). The influence of the shared environment is however, more modest explaining up to 40% of the variance in behaviour problems (61, 267-269).

Since the 1990s there has been an increased interest into the research of the effect of the intrauterine environmental and maternal well-being during pregnancy on later child development. Epidemiological studies suggest strong links between measures of the quality of the prenatal environment and the risk of cardiovascular and metabolic diseases (270, 271) and more research evidence suggests that low birth weight can be linked with impaired cognitive development and behavioural disorders, especially

hyperactivity/inattention (272, 273) while several studies have examined the association between low birth weight and internalizing and externalizing behaviours (274-276). Research findings (66, 245) suggest that maternal pre-pregnancy obesity/weight is associated with reduced cognitive abilities, symptoms of inattention and negative emotionality in school aged children (245). These findings are of clinical importance especially in the light of the increasing prevalence of obese women entering pregnancy (277). Pregnancy comes with main changes in the maternal body and a high pre-pregnancy weight is more likely to make these adaptations even more difficult affecting child development. This study aims at the investigation of the heritability of behaviour problems in a sample of young children between the ages of 18 months to 5 years old and the effect of maternal pre-pregnancy weight on children's behaviour problems.

8.2 Methods

The methods for recruitment have been previously described in Chapters 5 and 7. For this chapter twins aged between 18 months and five years old at the time of the survey were identified. In the time period between July 2008 and May 2010, 443 parents of eligible twins (434 (98%) mothers and nine (2 %) fathers) completed the study's online questionnaire on their twins' emotional and behavioural development.

8.2.1 Zygosity determination

For the determination of the twins' zygosity the previously adapted version of Goldsmith's zygosity questionnaire (See Appendix 3) was used (171). This questionnaire method of assigning zygosity has been validated against determination by

identity of polymorphic DNA markers and has reached accuracy in verifying zygosity in 95% of the cases (167).

8.2.2 Twin Sample

In total 886 twins were included in the analyses; 186 monozygotic (MZ) male twins, 138 monozygotic (MZ) female twins, 144 dizygotic (DZ) male twins, 158 dizygotic (DZ) female twins and 260 opposite-sex twins (132 male-female and 128 female-male twins).

8.2.3 Measures

Maternal pre-pregnancy weight

The primary exposure variable was maternal pre-pregnancy BMI, which was calculated as the weight (kg)/ height (m^2). Pre-pregnancy BMI was analysed both as a continuous and as a categorical variable. Pre-pregnancy BMI was classified according to the WHO standard guidelines as: underweight (<18.5kg) normal weight (18.5kg-24.99kg), overweight/obese (>=25kg).

Mothers of dizygotic twins had a higher gestational age, (36.22, 95 % CI: 36.00-36.43, p<0.001) compared to mothers of monozygotic twins (35.24, 95 % CI: 35.12-36.43); the monozygotic twins were older (3.13 years old, 95 %CI: 3.03-3.23, p<0.001) compared to dizygotic twins (2.91 years old, 95 %CI: 2.83-2.98) (See table 8.3).

The Child Behaviour Checklist (CBCL/1½-5)

The scale has been described in more detail in chapter 5. In brief, the Child Behaviour Checklist for toddlers (CBCL/1½-5) (181) is used to obtain standardised parent reports

of children's problem behaviours. It contains ninety-nine problem items, split into seven subcategories: emotionally reactive, anxious/ depressed, somatic complaints, withdrawn, sleep problems, attention problems, and aggressive behaviour originally derived by factor analyses (181). 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 behaviour. 'Total problems' is the sum score of all ninety-nine 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 2 months. Good reliability and validity have been reported for this checklist (181). To identify children that may be above the normal range for the syndrome scales children were categorized as being in the normal range when their T scores were below 65 (or the 93rd percentile) and as being in the borderline/clinical range when T scores$\geq$ 65 (or the 97th percentile) (181). For Internalizing, Externalizing, and Total Problem broadband scales the cut-off point used for the normal range was a T score <60, and borderline/clinical $\geq$ 60.

8.3 Statistical analysis

8.3.1 Correlations

Intra-pair twin correlations were calculated by using the Pearson's (r) and the Spearman's rho coefficient statistics as appropriate to explore the genetic and environmental influences.

8.3.2 Heritability analysis

Univariate genetic models were fit to the data in order to estimate the heritability of the problem scales using a maximum likelihood approach implemented in Mx (249). The estimates of the heritability are presented with 95% Confidence Intervals (CI) and goodness of fit statistics for several models: a full ACE model, in which the phenotypic variance is explained by genetic (A) common environmental factors (C) and non-shared (E) environmental factors. Reduced models were estimated by removing one of the parameters at a time and re-running the model. The goodness of fit of the reduced models was compared to the full model to assess whether they represented a better explanation of the data using the likelihood ratio χ^2 test and the Akaike Information Criterion (AIC). The models were assessed by examining the decrease in the fit of the model, if a parameter could be dropped without a significant decrease in fit then on the grounds of parsimony the reduced model was accepted as the best fitting model. Models were fit both unadjusted and after adjusting for maternal weight.

8.3.3 Regression analyses

Effects on a continuous scale

Standardized coefficients (Betas) are presented reflecting the change on the subcategories and the broadband problem scales by change in mother's weight both expressed as standard deviation change. In order to estimate the effect of the different categories of maternal weight on clinical problems, logistic regression models were fitted as described below.

Effects on clinically important behaviour problems

Logistic regression models were used where the intercept of each twin pair was modelled as a function of the population intercept plus the individual contribution of the twin pair. Associations of maternal pre-pregnancy weight and the syndrome and broadband scales were explored both unadjusted and adjusted for twins' sex, age, birth weight, maternal smoking (before, during and after pregnancy) and maternal educational level and gestational age. All analyses were performed in STATA 11 (220).

8.4 Results

8.4.1 Correlations

The means for the three broadband scales and the intra-pair twin correlations for MZ and DZ twins were calculated (Table 8.1). For Externalizing problems, the MZ correlation was r=0.89 and the DZ correlation was r=0.62. For Internalizing problems, the MZ and DZ correlations were r=0.81 and r=0.56 respectively. For Total problems, the MZ and DZ correlations were r=0.92 and r=0.75 respectively.

8.4.2 Heritability analyses

Variance estimates of ACE models and sub-models with their 95% Confidence Intervals (CI) are presented in Table 8.2, in which the most parsimonious model is highlighted. For Externalizing problems, an ACE model was the most parsimonious with genetic factors explaining 46% (95 % CI: 33-60%) of the variance, common environment explaining 42% (95 % CI: 27-54%) and non-shared environmental factors explaining 13% (95% CI: 10-16%) of the variance. For Internalizing problems a CE model was the most parsimonious model with common environment explaining 51% (95% CI: 44-58%)

of the variance and non-shared environment explaining 49% (95% CI: 42-56%) of the variance. For Total problems, an ACE model was the most parsimonious one with genetic factors explaining 26% (95 % CI: 13-39%) of the variance, common environment explaining 61% (95% CI: 49-70%) of the variance and non-shared environment explaining 13% (95% CI: 10-17%) of the variance.

After adjusting for maternal pre-pregnancy weight, the most parsimonious model for the Externalizing problems was an ACE model with genetic factors explaining 50% (95% CI: 36-68%), common environment 38% (95% CI: 20-52%) and non-shared environment 12% (95% CI: .09-16%) of the variance. For Internalizing problems the most parsimonious model was and AE model with genetic factors explaining 59% (95% CI: 50-67%) and non-shared environment 41% (95% CI: 33-50%) of the variance. For Total problems the most parsimonious model was an ACE model with genetic factors explaining 25% (95% CI: 14-38%), common environment 62% (95% CI: 49-72%) and non-shared environment 13% (95% CI: .10-17%) of the variance.

The mean maternal body mass index (BMI), gestational age (measured in completed weeks of gestation), age and sex of the twins stratified by zygosity are presented in Table 8.3. There were no differences in maternal weight between monozygotic and dizygotic twins. However, differences were observed between monozygotic and dizygotic twins with regards to gestational age and sex of twins. Correlations of maternal and twin covariates and problems broadband scales are also presented in the same table.

8.4.3 Regression analyses

In the unadjusted there was a highly significant increase of .08 standard deviations in aggressive behaviour with every standard deviation increase in maternal weight (p=0.02). The logistic regression analysis partly confirmed these findings. Overweight mothers were 1.10 times more likely to have a child with clinically aggressive behaviour when compared to normal weight mothers and .78 times more likely when compared to underweight mothers. The individual odds ratio (OR) did not reach statistical significance; a trend, however, (OR=1.10, 95% CI: .58-2.06) is apparent for children of overweight mothers to show clinically aggressive behaviour.

Similarly, there was an increase of .09 standard deviations (p=0.02) in externalizing problems with every standard deviation increase in maternal weight in the unadjusted model. An increase of the likelihood of externalizing (OR=1.32, 95% CI: .84-2.05) for children with overweight mothers compared to children of normal weight mothers was also apparent. No other statistical significant associations between maternal pre-pregnancy weight and behaviour problems were observed.

8.5 Discussion

In this study the influence of genetic and environmental factors on child behaviour problems and the effect of maternal pre-pregnancy weight on problem behaviours was investigated. The heritability analysis suggested that genetic and common environmental factors account for most of the variation in externalizing disorders, while common and non-shared environment explain most of the variation in internalizing disorders. After adjusting for mothers' weight there was a non significant decrease (of

2%) of the variation in the externalizing problems that can be explained by the common environment, suggesting that mothers' weight may play an important role in explaining externalizing problems.

In general, the results showed that children of overweight mothers showed a trend towards being more aggressive and exhibit externalizing behaviours compared to children of normal weight mothers. Aggressive behaviour is considered an important aspect of externalizing behaviour and has developmentally been linked to antisocial behaviour (278, 279). Studies with children focusing on aggression, have shown that both observed aggression and parental reports of externalizing behaviours are relatively stable from toddlerhood to five years and beyond (40, 280), which may highlight the influence of genetic influences. Consistent with this are the results of this study which suggest that genetic factors can explain a large part of the variation in externalizing and internalizing behaviour problems.

The results from the logistic regression however, do not suggest a strong association between maternal overweight and behaviour problems. Moreover, the significantly observed change in the standardized coefficients could not explain the distinction between normal range and borderline/clinical range. Therefore, children appeared to be more aggressive but it is not clear whether this is of clinical significance.

Previous studies trying to identify the role of external factors in the development of behaviour problems have focused on the role of maternal weight. Rodriguez and colleagues (66, 245) showed that children of pre-pregnancy overweight mothers were more likely to show inattention symptoms and have difficulties with emotion intensity

and regulation based on parent and teacher reports. In this study the effect of mothers' weight on children's behaviour problems with a focus on externalizing and internalizing problems was investigated.

The role of nutrition on the development of the brain has been investigated in the past. Although there is not a clear pathway that links externalizing problems to maternal overweight, several pathways have been proposed to explain this association and parallels between overweight and other developmental disorders can be drawn. Leptin, which is the protein produced by adipose cells, has been found to play multiple functions in reproduction (281, 282), glucose homoeostasis (283, 284) as well as in brain (285, 286) and neurocognitive development (287). Another possible causal pathway suggests that pre-pregnancy overweight women may not be able to synthesize vitamin D, due to the excessive adipose tissue which results in deficiency in both the mother and the neonate; in turn, vitamin D is associated with neurocognitive function (288). In addition, high glucose levels pose a risk for neurobehavioural impairments (289).

However, others have emphasized the importance of the dynamics within the family environment, such as the parent-child interaction and the individual differences in parenting (290, 291) and their impact on the course of the developmental pathways of problem behaviours. Previous research has mainly linked externalizing problems with family adversity, maternal depression and low socioeconomic status (283, 290, 292).

In addition, there may be other environmental factors that may accentuate these symptoms that wasn't possible to be examined through this study. For instance, parents with children displaying symptoms of aggressive behaviours normally report higher levels of stress and frequent use of negative parenting strategies (293) while it has been found that stress levels are associated with weight gain (294). Thus, parental behaviour may fuel noncompliance, aggression and poor regulation of emotion, rather than providing toddlers adaptive models of regulated and prosocial functioning (295).

To sum up, these results suggest a possible association between aggressive/externalizing problems and maternal overweight. Previous studies have reported a strong association between overweight mothers and inattention but this study did not replicate this. It is important to keep in mind when conducting research with preschoolers that the investigation of children who may be at risk for externalizing problems, may pose the challenge of differentiating between age-related and normative levels of this behaviour from more serious early-emerging problems (38).

Table 8.1 Descriptive statistics of problem broadband scales for monozygotic and dizygotic twin pairs

	Monozygotic twin pairs							Dizygotic twin pairs						
	Twin 1			Twin 2				Twin 1			Twin 2			
Problem scales	N	M	SD	N	M	SD	r	N	M	SD	N	M	SD	r
Externalizing (range 0-48)	167	12.88	7.72	166	12.83	7.51	.89	269	12.79	6.61	268	12.66	6.95	.62
Internalizing (range 0-96)	167	5.94	4.55	166	9.13	6.98	.81	269	5.49	4.11	268	8.24	5.89	.56
Total problems (range 0-78)	167	31.58	16.24	166	34.66	18.90	.92	269	30.45	14.18	268	33.06	16.10	.75

N; Number of twins
M; Mean
SD; Standard deviation
r; Within-twin correlations

Table 8.2 Univariate genetic model-fitting for the problem scales presenting full and nested models

	A (95%CI)	C (95%CI)	E (95%CI)	Δx^2	P	AIC
Externalizing						
ACE*	.46 (.33-.60)	.42 (.27-.54)	.13 (.10-.16)	-	-	-
AE	.87 (.84-.90)	(0)	.13 (.10-.16)	23.44	.00	21.44
CE	(0)	.74 (.69-.78)	.26 (.22-.31)	49.69	.00	47.69
Internalizing						
ACE	.23 (.00-.48)	.35 (.12-.54)	.43 (.35-.53)	-	-	-
AE	.60 (.52-.67)	(0)	.40 (.33-.48)	8.84	.00	6.84
CE*	(0)	.51 (.44-.58)	.49 (.42-.56)	3.15	0.07	1.15
Total Problems						
ACE*	.26 (.16-.39)	.61 (.49-.70)	.13 (.10-.17)	-	-	-
AE	.87 (.84-.90)	(0)	.13 (.10-.16)	53.64	.00	51.64
CE	(0)	.79 (.76-.83)	.21 (.17-.24)	22.17	.00	20.17

* Best fitting model

A, Additive genetic; C, shared environment; E, non-shared environment; CI, confidence interval; $\Delta\chi^2$, Difference Chi-square; P, statistical significance p<.05; AIC, Akaike's Information Criterion

Table 8.3 Means, standard deviations and Pearson/Spearman correlations with three syndrome scales for each covariate, stratified by zygosity

	MZ twins					DZ twins				
Maternal Characteristics	**Mean**	**SD**	**r_a**	**r_b**	**r_c**	**Mean**	**SD**	**r_a**	**r_b**	**r_c**
BMI	24.51	4.32	.07	-.03	.02	24.76	5.10	.10*	-.04	.10*
Gestational age (weeks)	35.38	2.49	-.04	-.12*	-.06	36.22	2.71	-.04	-.15***	-.11**
Smoking (no/yes)^	**n**	**%**	**r_a**	**r_b**	**r_c**	**n**	**%**	**r_a**	**r_b**	**r_c**
Before	133/40	76.9/23.1	0.03	-0.02	0.05	255/54	82.5/17.5	0.07	0.04	0.06
During	156/5	96.9/3.1	0.07	0.00	0.06	280/10	96.6/3.4	0.10	-0.03	0.05
After	143/18	88.2/11.2	0.04	0.04	0.10	272/29	90.4/9.6	0.09	0.00	0.06
Educational level										
High School diploma or less	42	25.3				53	18.1			
College/professional education	23	13.9	-0.02	0.04	0.00	51	17.3	-0.00	0.03	-0.03
University	101	60.8				191	64.6			
Twin Characteristics										
Age (years)	3.13	.96	-.23***	.02	-.15**	2.91	.95	-.07	.11	-.01
Sex	**n**	**%**	-.05	.18***	.07	**n**	**%**	-.08	-.05	-.09*
Male	206	57.22				164	26.28			
Female	154	42.78				174	27.88			
Opposite sex	-	-				286	45.84			

^Smoking refers to smoking before, during, after pregnancy
r_a Pearson/Spearman correlation with Externalizing scale
r_b Pearson/Spearman correlation with Internalizing scale
r_c Pearson/Spearman correlation with Total Problems scale
*p<.05; **p<.01; ***p<.001

Table 8.4 Logistic and linear regression for children's behavioural problems based on maternal BMI

	Behavioural problems	No behavioural problems	OR	95% CI	Beta	P	OR**	95% CI	Beta	P
Emotional reactivity**	N	N			.03	.42			.02	.58
Normal weight	25	376	1.00	Reference			1.00	Reference		
Underweight	6	82	1.10	.44-2.77			1.12	.42-2.96		
Overweight/Obese	20	283	1.06	.58-1.95			1.05	.54-2.06		
Anxiety/Depression**	N	N			-.03	.40			-.02	.62
Normal weight	29	372	1.00	Reference			1.00	Reference		
Underweight	5	83	.77	.29-2.06			.86	.31-2.41		
Overweight/Obese	14	289	.62	.32-1.20			.65	.32-1.30		
Somatic Complaints**	N	N			-.01	.11			-.07	.07
Normal weight	39	362	1.00	Reference			1.00	Reference		
Underweight	11	77	1.33	.65-2.70			.99	.43-2.26		
Overweight/Obese	30	273	1.02	.62-1.68			.99	.56-1.75		
Withdrawn behaviour**	N	N			-.06	.09			-.02	.58
Normal weight	12	389	1.00	Reference			1.00	Reference		
Underweight	3	85	1.14	.32-4.14			1.53	.36-6.61		
Overweight/Obese	14	289	1.57	.72-3.45			2.66	.99-7.09		
Attention**	N	N			.06	.08			.03	.39
Normal weight	30	371	1.00	Reference			1.00	Reference		
Underweight	5	83	.74	.28-1.98			1.17	.42-3.29		
Overweight/Obese	29	274	1.31	.77-2.23			1.13	.60-2.13		
Aggressive behaviour**	N	N			.08	.02[a]			.07	.07
Normal weight	23	378	1.00	Reference			1.00	Reference		
Underweight	4	84	.78	.26-2.32			.87	.28-2.69		
Overweight/Obese	19	284	1.10	.58-2.06			1.04	.52-2.10		
Sleeping problems**	N	N			.06	.11			.03	.48
Normal weight	14	387	1.00	Reference			1.00	Reference		
Underweight	3	85	.98	.27-3.47			.97	.20-4.66		
Overweight/Obese	11	292	1.04	.47-2.33			.92	.37-2.29		
Externalizing	N	N			.09	.02[a]			.07	.08
Normal weight	46	348	1.00	Reference			1.00	Reference		
Underweight	6	81	.56	.23-1.36			.66	.26-1.66		
Overweight/Obese	44	253	1.32	.84-2.05			1.17	.69-1.99		
Internalizing	N	N			-.04	.29			-.03	.44
Normal weight	43	358	1.00	Reference			1.00	Reference		
Underweight	16	72	1.85	.99-3.46			1.86	.93-3.73		
Overweight/Obese	34	269	1.05	.65-1.70			1.01	.59-1.74		
Total problems	N	N			.07	.05			.06	.14
Normal weight	44	355	1.00	Reference			1.00	Reference		
Underweight	6	82	.59	.24-1.43			.59	.23-1.50		
Overweight/Obese	41	260	1.27	.81-2.00			1.18	.70-2.00		

** Categorized based on children within normal range and borderline/clinical range **Adjusted for gestational age, twins'birth weight, age and sex, mother's educational level, smoking (before, during, after pregnancy)* [a] $p<0.05$

CHAPTER 9

GENERAL DISCUSSION

Paragraph 9.5: Antoniou EE, Zeegers MP. The advantage of using twin studies in epidemiological research. British Journal of Obstetrics and Gynaecology, 2011. In press

9.1 General Discussion

9.1.1 Summary of findings

In this thesis the results from the analysis on two twin samples exploring different pathways of the influence of intrauterine environment markers on child cognitive (IQ) and behavioural development were presented. For the study on cognitive development and intrauterine environment the genetic and environmental correlations between intrauterine factors and IQ were investigated. First, the influence of birth weight, placental weight and morphology and umbilical cord knotting, length and insertion on later cognitive development (Chapter 3) was studied. The results confirmed previous findings on high heritability estimates of IQ (60-74%) and extended the research evidence supporting a possible link between birth weight and IQ. An increase of 100gr of birth weight resulted in an increase (of .38 points) on total IQ while twins with knotted umbilical cords had lower (1.92 points) total and verbal IQ points (1.70) compared to twins with no knots. The genetic analyses suggested that the etiology of IQ is distinct of that of birth weight and cord knotting and that non-shared environmental factors may influence this relationship.

These findings highlighted the importance of the umbilical cord and its impact on later outcomes and subsequently lead to the investigation of the genetic and environmental aetiology of the umbilical cord's morphology and pathology (Chapter 4). Therefore, the heritability of the umbilical cord's length, knotting, twisting, number of vessels and type of insertion on the placenta were investigated. The twin study design and the information on the placental characteristics of the twins facilitated the examination of

the genetic and environmental influences on the cord based on the twins' zygosity and chorionicity.

Overall, the results suggested that genetic and environmental factors influence the formulation of cord length and knots, and it can be speculated that monozygotic twins may be exposed to a more adverse intrauterine environment compared to dizygotic twins. For cord insertion, twisting and number of vessels, environmental factors proved to be more important. Certain conditions *in utero*, which are discussed below, may determine the site of the insertion on the placenta, which have no genetic predisposition and may involve the sharing of one placenta or the unique motor activity of each twin *in utero*. Broadly, these findings can inform research about the intrauterine environment status and the way it could affect later development.

Apart from the fetal influence, maternal prenatal influences can affect children behavioural problems later in life (296, 297). In this thesis the heritability of very young's children temperamental characteristics and emotional and behavioural problems and the influence of maternal pre-pregnancy weight on these traits were investigated. Previous findings (66, 245) suggested that children of overweight mothers are more likely to have emotional problems and show inattention symptoms. The results suggested that children of pre-pregnancy overweight mothers did not have any differences in their temperamental characteristics compared to children of normal weight mothers (Chapter 7). However, there was a significant trend observed for the children of overweight mothers who showed more aggressive/externalizing behaviours compared to children of normal weight mothers (Chapter 8).

9.2 The Developmental Origins of Health and Disease hypothesis (DOHaD)

The developing fetus is considered to be protected by the environment. However, evidence suggests that the fetus responds to the environmental factors with consequences pertaining to changes in the structure, physiology of the fetus and later development (298). This process known as fetal programming or developmental plasticity is one of the core assumptions of the DOHaD hypothesis (299-301). In some cases these changes can be adaptive, preparing individuals that are best suited to the environment forecast by the clues available in early life (302). In other instances the effect of the early environment results in non adaptive changes as a possible result of physical or chemical constraints disrupting developmental processes.

Previous studies investigating this hypothesis have revealed an inverse association between birth weight and physiological outcomes, such as coronary heart disease (303), hypertension (304), insulin resistance (305) and stroke (306). Recent epidemiological studies have sought to evaluate the effect of specific *in utero* factors, such as smoking, stress, nutrition, low birth weight on subsequent risks in child development (252, 307). This approach will indicate whether interventions to improve maternal and child health and development could have long-term benefits.

Some of the relavant factors are related to mother's behaviour before or during pregnancy, in particular nutrition, smoking and drinking alcohol, as well as with stressful events in the mother's environment. These factors have consequences for the fetal environment either indirectly by altering the oxygen and nutrition supply to the

fetus or directly by transfer of maternal agents across the placenta (308) and are associated with fetal growth. Psychosocial stress (307) and smoking during pregnancy (309) (252) can affect fetal growth and later development. Below, the results from each chapter regarding fetal and maternal influences are discussed.

9.3 Prenatal factors- Intrauterine environment

9.3.1 Birth weight and umbilical cord knotting associations with IQ

The difficulty to obtain reliable data about the nature of the fetal environment in human studies have lead many epidemiological studies to use measures on birth outcome such as birth weight. Size at birth is the product of a fetus's pattern of growth, which is apparent at an early stage in development, and the maternoplacental capacity to supply sufficient nutrients and oxygen to maintain this pattern (310). The results in Chapter 3 confirmed previous research findings suggesting a link between IQ and low birth weight (28, 31, 97, 131) (33) within the DOHaD hypothesis. This study has shown that genes may partly and moderately explain the relationship between birth weight and IQ.

Nevertheless, the wide confidence intervals of the correlations and the finding that the contribution of genetic factors to variation in birth weight was relatively small, suggest that the association is unlikely to be attributed to genetic factors and that these results should be interpreted with caution. Despite the fact that an adverse intrauterine environment is a first expression of a genotype that is expressed as a cognitive phenotype in later life, there seems to be some environmental influence explaining the associations between birth weight and IQ.

Because of the large number of environmental factors that could influence the relationship between birth weight and IQ, it is difficult to know which factors in particular mediated by birth weight may affect developmental vulnerability in later life. Possible pathways affecting the link between birth weight and IQ have been suggested. Previous research has shown that IGFs play a critical role in determining overall body growth (139) while IGFs have also been found to be responsible for the development of parts of the brain, like memory and learning, a finding that could perhaps explain the link between body size and later cognitive development (311). Prenatal stress (312) and stress during pregnancy have also been associated with low bithweight (313) while, in a large longitudinal study, Laplante and his colleagues (314) found that high levels of prenatal stress exposures may negatively affect the brain development of the fetus, reflected in the lower general intellectual abilities. In addition, findings of maternal high anxiety levels during pregnancy are associated with both lower birth weight and later impaired cognitive functions, such as recognition and memory (315).

The examination of the effect of the umbilical cord morphology on IQ lead to the significant and novel finding that twins with knotted cords had lower total and verbal IQ scores compared to twins with no knots. Many aspects of the umbilical cord morphology have been associated with adverse physiological outcomes (24, 142, 143) but the pathway to cognitive outcomes remains unclear. The findings of this thesis suggest that a knotted cord can have an impact on later cognitive development. A possible mechanism behind this association may suggest that because nutritional status *in utero* is very important for later development, anything that constricts the normal flow of nutrients and oxygen can have an unfavourable outcome (119, 120).

Another possible explanation on the link between knotting and lower IQ can be given by previous studies which have shown that cord knots are responsible for causing hypoxia in the fetus (316), while in a review article by Bass and colleagues (317) it has been suggested that hypoxia and even mild levels of oxygen restriction can have an impact on later cognition.

9.3.2 Umbilical cord morphology

The implementation of the twin design facilitated the investigation of the influence of genetic and environmental factors on the aetiology of the umbilical cord morphology and pathology based on zygosity and chorionicity (Chapter 4). There is growing evidence suggesting that all aspects of the studied umbilical cord characteristics in this thesis have been linked to pathological outcomes (25, 142, 143, 318). The umbilical cord is one of the most vital parts of the human anatomy since it is the pathway between the mother and the fetus that is responsible for the nutrients and oxygen supply necessary for life. The investigation of the pathological characteristics of the cord gives an insight into the intrauterine environment, the fetal growth and the cord's impact on later development. The heritability analyses performed in Chapter 4 suggested that genetic and environmental factors influence the development of cord length and knots highlighting the assumption that there may be a genetic predisposition in the amount of fetal movement in utero, while a more adverse environment due to the twins being raised in the same chorion or amnion may impact on different experiences of the twins *in utero*. Cord insertions, number of vessels and twisting were mainly influenced by environmental factors. This finding pinpoints the individual's intrauterine circumstances which could affect the site of the implantation on the placenta and the development of

number of vessels. The aetiology of cord twisting however, has been a debatable matter, since one theory (25) suggest that there is a genetic difference in the direction of the fibres in the vessel walls that could affect the cord twist, while another theory (26) suggests that each twin may experience an area low in Wharton's jelly, which results in less resistance from the fetus and therefore causes twisting of the cord. Although a twin design was used with a main focus on the differences between monochorionic twins who may be eventually more compromised in their upbringing compared to dichorionic twins, evidence from case studies on singletons suggests that the umbilical cord functioning is very important since pathologies of the cord have been linked to adverse outcomes in singleton pregnancies as well (118).

9.3.3 Maternal pre-pregnancy weight and behaviour development

So far, the main prenatal environmental factors which have been taken into account are factors related to the fetal birth weight. Although the maternal influences, especially within the intrauterine environment, are difficult to distinguish from the fetal influence on later outcomes, previous research has focused on the association between maternal pre-pregnancy weight and inattention (245). Based on these findings, the research in this thesis was focused on the association between maternal pre-pregnancy weight and child temperament and behaviour development.

In this thesis no association was found (Chapter 7) between the two factors. However, the investigation of the association between the maternal weight and behavioural problems (Chapter 8) suggested that there may be a trend for children of overweight

mothers to show more aggressive and externalizing symptoms compared to children of normal weight mothers.

The role of nutrition on brain development has been the subject of previous research (246). As has been mentioned previously in the discussion of the findings in Chapter 8, there are no clear pathways linking externalizing problems to maternal weight. However, some mechanisms implicating leptin and vitamin D are discussed in relation to other developmental disorders. Leptin is associated with overweight and obesity (285) and is necessary for optimal brain development (286). Although improved cognition is associated with leptin, increased risk of intellectual disability in children is seen in those with pre-pregnancy obese mothers (319). In a review of studies by McCann and colleagues (320), in which they examine the effects of vitamin D on brain dysfunction, they point out that evidence for vitamin's D involvement in brain function includes the wide distribution of vitamin D receptors throughout the brain. They also discuss vitamin's D ability to affect proteins in the brain known to be involved in learning, memory and motor control.

9.4 Influence of the intrauterine environment on psychological and physical development

This thesis focused on the investigation of behaviour and cognitive development in young infants and children. To put this into context, in the following paragraphs, a more comprehensive approach focusing on the impact of the intrauterine environment on older children and adult development is taken.

9.4.1 Early life origins of cognitive development in young and adult life

Previous findings from longitudinal birth cohort studies (15, 321) have emphasized the importance of smaller body size at birth in predicting low cognitive functioning in later life. However, it is not clear which of the human growth periods, when examined up to adulthood, are the most critical in terms of cognitive development (243). Measures on birth weight, monthly measurement of weight change and cognitive assessment of verbal, arithmetic and visuospatial abilities at the age of twenty years in men from the Helsinki Birth Cohort Study (HBCS) allowed the examination of the critical periods of growth from birth to twenty years for cognitive development (322) adjusting for familial factors. Slower growth and weight gain between birth and two years predicted poor performance in the tests. In addition, birth weight and slower linear growth between eleven and twenty years predicted worse performance in the three tests. These findings suggest that slow prenatal brain growth and slow linear growth to two years after birth could form a first critical period for intellectual development. The second critical period may be present during adolescence. In line with the finding that a second critical period may be during adolescence are the results of this thesis, which showed that low birth weight is associated with lower IQ scores in children between seven to fifteen years old. Other studies assessing the effect of birth weight on cognitive function throughout the lifecourse have found that the positive association between birth weight and cognitive function seems to decline with age. In the UK 1946 birth cohort study, birth weight was linked to cognitive ability at age eight, was maintained during adolescence and early adulthood but was much weaker in midlife (when looked at age forty-three) (131). Other studies have also shown a weak association between birth weight and cognitive

function in midlife (323). It has been suggested that adult environmental influences, such as educational and occupational attainment may overshadow perinatal factors by midlife (131)

9.4.2 Early life origins of temperament in young and adult life

The concept of temperament is based on the assumption that there are stable individual differences in behaviour, reactivity and self-regulation and that these differences are due to innate biological factors but can also be influenced by experience or maturation. Previous studies have mainly focused on the association between birth weight and development of temperamental characteristics in different age groups employing various research designs and questionnaires. Restricted fetal growth has been associated with activity level and duration of orienting in six months old infants (324). Nevertheless, this association was not significant after adjustment of factors that indicate genetic influence (e.g. maternal height) or common environmental influences (e.g. socioeconomic status). In this thesis, when maternal weight was entered in the genetic models (Chapter 7) it did not have any effect and therefore could not explain any of the variation of the temperamental dimensions. Even though temperament is thought to be a biological trait many aspects of it, such as activity level and distress to limitations are highly heritable.

Associations between length at birth and ponderal index with negative affectivity have been reported in five year old children (16). Inverse associations of fetal growth with

effortful control have been reported in a study (276) but no associations were observed with extraversion/surgency or negative affectivity. In addition, the same study showed that effortful control mediated the association between fetal growth and hyperactivity/inattention, suggesting a potential pathway via temperament, which may also become a vulnerability factor for behavioural problems. In a cohort study of over sixty years old adults, Raikkonen and his colleagues (325) found negative associations between birth weight and hostility, in particular in participants with very low birth weight (<2.5kg). Taken together, these studies show that prenatal environmental factors affect the development of temperamental traits and may suggest that the DOHaD-framework of early origins may offer an insight into the individual differences in temperament.

9.4.3 Early life origins of behaviour problems in young and adult life

As already previously established, events occurring in prenatal life may have lasting effects on behaviour and health (302). Fetal growth, indexed by birth weight, has been shown to have an impact on the development of emotional and behavioural disorders later in life. However, many important prenatal risk factors that impact on child development *in utero* are influenced by maternal characteristics.

Maternal nutrition during pregnancy, smoking (326, 327) and prenatal stress (328, 329) have been linked to several behavioural alterations including poorer attention regulation and more difficult temperament. In this thesis, the results from the study on the association between externalizing and internalizing problems and maternal pre-

pregnancy weight (Chapter 8) confirmed previous studies suggesting that mothers' weight before pregnancy was linked to children's aggressive behaviour.

Results from the Helsinki Birth Cohort study (330) examinining whether the effects of pre- and post-natal growth up to eleven years of age and in adulthood are associated with anxiety at the age of 63 years old showed that lower birth weight, smaller BMI and smaller head circumference at birth was associated with higher trait anxiety. Higher anxiety was not solely related to smaller body size at birth and slower prenatal growth, but individuals who had scored high in trait anxiety remained lighter in weight and were thinner throughout infancy and put on weight more rapidly from seven to eleven years. However, they continued to weigh less and were shorter than others in late adulthood. This pattern of growth can be compared to the pattern of growth of somatic disorders in adulthood (10). These findings suggest that a large influence on developing anxiety may be programmed early in life. As many prospective epidemiological studies have linked anxiety to somatic diseases and more specifically, cardiovascular disease, these findings may suggest that anxiety and somatic diseases may share a common origin in an adverse prenatal and childhood developmental trajectory. The twin study designs are a valuable tool in examing the genetic and environmental influences and investigate the role of the intrauterine factors and have been widely used in epidemiological research.

9.5 Why was an online twin study conducted?

Traditionally, aetiological epidemiological research has been using the following templates of research design: a) controlled clinical trials, which produce the most

scientifically robust outcomes as this design usually gives control over the exposure, tries to match comparison groups, and allows for the passage of time between exposure and outcome measurement; b) cross-sectional study that cannot control but can measure the exposure factor and outcome at the same time; and c) observational study, including the case-control and cohort studies that can only measure exposure and outcome but does allow for the passage of time between exposure and outcome measurement. A shared quality of these templates is that their unit of analysis is the individual subject.

The twin study design, which was used in this thesis, may offer a different approach in which statistical analyses can take place on both the individual and on the twin pair level. In this twin study, the data analysis was performed from data obtained from monozygotic (MZ) and dizygotic (DZ) twins raised in the same family environment. As MZ twins inherit the same genetic makeup and DZ twins share half of their genes, their variation in health outcomes can be separated into genetic, familial and individual influences, in addition to investigating the relationship between exposure and health outcome. The calculation of intra-pair correlations which was done in all of the chapters of this thesis or the use of Structural Equation Modelling (SEM) enabled the estimation of the heritability of the phenotypes under study. The association between exposure and outcome can be analysed on the twin pair level in which the difference in exposure between two twins is compared to their difference in the health outcome under study. Often, in twin research binary health outcomes are used and this method is called co-twin control analysis (331). Differences in results between these two methods will allow the examination of confounding by familial influences, whereas differences in results between MZ and DZ twins show confounding by genetic factors. In Chapters 3, 4, 7 and

8 where a heritability analysis on the different outcomes was performed, phenotypes that have genetic aetiology can be distinguished from the phenotypes that have shared familial aetiology. For instance, high heritability estimates of IQ scores lead to the conclusion that IQ is genetically determined, whereas largely shared environmental factors influence the development of duration of orienting for infants' temperament. Similarly, one could assess whether comparable health outcomes are being influenced by the same of different genetic or familial influences in bivariate SEM models (71) or whether the heritability of certain health outcomes are determined by third factors in gene-environment interaction models (332). In Chapter 3 a bivariate analysis was performed to investigate the correlation between birth weight and IQ and the correlation between umbilical cord knots and IQ. The results suggested that these two phenotypes do not share the same genetic or environmental aetiology and non-shared factors may influence their relationship.

9.6 The use of internet studies in epidemiological research

With the Internet becoming increasingly accessible, the distribution of online studies has already been an established tool for conducting research within the epidemiological field (333). The use of electronic surveys has undoubtedly some advantages but also some disadvantages. A main advantage of using the web is the access it provides to a large population of individuals. Online surveys can benefit from the fact that they can be administered by the use of interactive forms. The web-based technology provides an inexpensive mechanism for conducting surveys online instead of using postal mail, with the advantage of low cost and quick distribution. In addition, electronic surveys provide

the ability to transfer responses directly into a database, reducing this way, transcription errors. Although questionnaire screen design is developed in HTML (hypertext markup) which makes the design more complex, web-based suveys provide format and response control. For instance, for this thesis, one-answer radio buttons questions were used which prevented multiple responses, and coded and open-ended questions were also accommodated in the design.

While web-based surveys have multiple advantages, they also have some disadvantages. It is important when conducting an online survey to be cautious of possible sources of error and ethical issues. A possible source of error in web surveys is the sampling error, arising from the fact that people who do not have access to the web do not have the same chance to take part in the study compared to those who have access (334). For this thesis, the goal of the recruitment strategy was not to systematically recruit all parents and twins in UK but to establish a volunteer-based database of parents and their twins. Even though the invitation letters were distributed to most members of the twin clubs is was not possible to estimate who decided not to take part.

Another disadvantage of the web based surveys is an increased probability of nonresponse bias, which arises when respondents to a survey are different from those who did not respond in the survey (335). Based on a survey conducted by TAMBA at the time of the recruitment for this study, the sample characteristics were not different from those presented in this thesis.

From another point of view, ethical concerns have been put forward regarding data security (335). To protect anonymity and confidentiality in this study the data were secured and the respondents reassured that the data they provided are securely stored. However, the advantage of web surveys to use hypertext markup and include features that can enable the completion of questionnaires, such as links to further information on the study, was utilized in this thesis in order to investigate the amount of information people needed to read before they decided to participate in this research by using expandable information (Chapter 6).

9.7 Conclusion and implications of the study

Previous research on early life origins of psychological development and mental health agree well and lend credence to the DOHaD hypothesis. Therefore, it is very important not only to study physical growth as an aetiological factor of somatic disorders but also as a determinant of psychological vulnerability factors for mental disorders. This is particularly important since evidence from twin studies suggest that the impact of developmental programming is not to be restricted to disorders, but may extend to include a range of temperamental or behavioural dispositions independently of genetic effects (20).

Possible explanations within the DOHaD framework (336) have been suggested relating mainly low birth weight to later outcomes. The results of this thesis suggest that maternal pre-pregnancy weight and the influence of the umbilical cord pathology are

also very important intrauterine markers that should be further investigated in relation to later behaviour outcomes that could affect children well-being.

Other important factors to be considered involve aspects of the extrauterine environment, which may be apparent later in life and could affect development interacting with the intrauterine factors.So far, observational and experimental evidence suggests a relation between growth and development during fetal and young child life and health in later years. This association may have some implications. First, it strengthens the growing understanding that investment in health and education of people in relation to their responsibilities during pregnancy is very important. An approach to health care should focus on a life course perspective. The World Health Organization has recognized the importance of this approach in their guidelines on diet and nutrition in order to promote optimal fetal development. The outcome of a pregnancy should be considered in terms of fetal, maternal and the neonatal health and also alongside the cognitive, emotional and behavioural development in the later life-course. Interventions could involve improvement of maternal diet before and during pregnancy if awareness on the adverse outcomes of an imprudent diet is increased. In the light of the evidence that umbilical cord pathology have an impact on later life course more detailed perinatal examinations of the utero should be undertaken in order to prevent adverse outcomes.

Novel trial designs within a socially and culturally appropriate context would help educate people about the necessity to modify their behaviour in favour of their children's later development. In addition, epidemiological birth cohorts are needed in

order to test the assumptions of the DOHaD hypothesis, especially when taking into account children that have not suffered major pre- and perinatal complications that may confound the effects of early growth on subsequent psychological development and mental health.

Models based exclusively on nutrition and central nervous system (CNS) pathways do not provide sufficient information on how nutritional deficiencies or nutritional abudance translate into maternal and child psychological/psychiatric development. Further research needs to incorporate nutrition-CNS pathways as well as pathways looking also at socially relevant maternal characteristics such as maternal stress, depression, contextual characteristics such as child rearing patterns, and individual characteristics, such as child temperament. Models including brain, context and child and maternal characteristics are more complex but they can offer more precise information of the processes involved in nutrition-psychological/psychiatric health relations (246).

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An empirical study on the preferred size of the participant information sheet in research

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ABSTRACT

Background Informed consent is a requirement for all research. It is not, however, clear how much information is sufficient to make an informed decision about participation in research. Information on an online questionnaire about childhood development was provided through an unfolding electronic participant sheet in three levels of information.

Methods 552 participants, who completed the web-based survey, accessed and spent time reading the participant information sheet (PIS) between July 2008 and November 2009. The information behaviour of the participants was investigated. The first level contained less information than might be found on a standard PIS, the second level corresponded to a standard PIS, and the third contained more information than on a standard PIS. The actual time spent on reading the information provided in three incremental levels and the participants' evaluation of the information were calculated.

Results 77% of the participants chose to access the first level of information, whereas 12% accessed the first two levels, 6% accessed all three levels of information and 23% participated without accessing information. The most accessed levels of information were those that corresponded to the average reading times.

Conclusion The brief information provided in the first level was sufficient for participants to make informed decisions, while a sizeable minority of the participants chose not to access any information at all. This study adds to the debate about how much information is required to make a decision about participation in research and the results may help inform the future development of information sheets by providing data on participants' actual needs when deciding about questionnaire surveys.

Gaining consent is a prerequisite for nearly all health research involving human participants. The main aim of gaining informed consent is to respect and promote participants' autonomy and to protect them from ignorance about potential harm. European directive regulations[1] stipulate that participants in clinical trials must be adequately informed about the aims, the method, the expected outcomes and the potential risks associated with study participation. It does not, however, elaborate on what 'adequately informed' amounts to in practice. Jefford and Moore[2] suggest that informed consent requires the provision of unbiased, up-to-date, relevant information on the consequences of choices, and that the potential participant can freely choose between two or more options (as a minimum whether to enter the study or not). However, they also do not specify the level of detail a potential participant needs to make a choice between the options offered. Current National Research Ethics Service (NRES) guidance suggests that, when appropriate, the participant information sheet (PIS) should be divided into two parts. The first part should contain brief and clear information on the essential elements of the specific study, such as what is the research about and what participants will have to do. The content of this part should be enough for participants to decide whether they wish to participate in the study. A second part should contain more detailed information, such as data confidentiality, which patients may wish to have.[3]

Some studies aimed at improving the readability of the information sheet have concluded that understanding might be improved if the form is easy to read,[4–6] and emphasise the need for plain, accessible language,[7–9] whereas others suggest that understanding and even recall of the information might be enhanced if sufficient time is allowed for reading.[10 11] Short consent forms may also be useful.[5 12 13]

The extent to which current practice ensures that adequate information is given to potential participants is unclear. Ferguson[14] found that most patients who participated in clinical trials did feel adequately informed and that they were capable of understanding most of the information provided. Similarly, Olver *et al*[15] found that out of 100 cancer patients, 68 felt they had been given the right amount of information, 14 felt there was insufficient information, and only five felt they received too much information. We were unable to find any research that empirically and systematically determined the actual amount and type of information people wanted in order to make a decision about participation in research—existing literature only reports on whether, having been provided with a fixed and predetermined amount of information, participants felt informed.

Not everyone is the same, and some people will want more, and some less, information than others. One of the advantages of web-based information is that it can use hypertext markup to make the text interactive and thereby enable users to choose what they want to see and access different levels of information according to their interests and needs. In an online survey-based study on childhood development, we provided the PIS in a structured format so that people could get the amount of information they felt they needed. We judged this method of presenting information was safe because the project posed little or no risk to the

participants; no clinical interventions or tests were involved and participants were asked to complete a questionnaire, which could readily be discontinued at any point and the information already recorded discarded. Therefore, the risk that participants might be harmed by entering a study on the basis of too little information was negligible.

This study sets out to explore how people used the information provided in order to inform the future development of information sheets according to participants' actual needs.

METHODS

The study reported in this paper investigates what information people sought or wanted in order to be able to decide whether to participate in an online study. This research was embedded within a nation-wide population-based study investigating the development of twins during early childhood, conducted by the University of Birmingham,[16] which involved the completion of an electronic questionnaire accessed via the study's website by a parent. The Ethical Review Committee of the University of Birmingham approved this study.

The population sampling frame for the study consisted of parents with twins aged from birth to 5 years of age. Advertisements for recruitment about the study were sent in the form of an invitation letter to members of the Twins and Multiple Births Association and via public advertisements in twin-specific magazines and websites. The final sample for the study (n=552) consisted of parents who completed the twin survey, and therefore had access to the PIS, between July 2008 and November 2009. We tracked what information was accessed and for how long using each participant's computer's internet protocol. Because this study was embedded within a larger study, demographic information was available to enable us to compare the information potential participants actually accessed before deciding to participate by various characteristics (see below).

Before completing the main survey participants were directed to a PIS, which offered access to six domains of information in three levels of detail. The domains provided answers to the following questions, which at the time of the study's design were recommended by the UK National Research Ethics Service:[3] (1) What is our research about?; (2) Why are we doing this study?; (3) Why have you been invited to take part?; (4) What would we like you to do?; (5) Who will see the information that is collected?; (6) What will happen to the information that is collected?

To access the information the participant had to click a (+) sign option next to each question. The first level of information was sufficient to give them a broad understanding of the nature of the project and what would be required of them if they chose to participate. The remaining levels were accessed by a deliberate decision of the potential participant by clicking on a second and then a third (+) sign option. The second level was longer and more detailed than the first and provided the reader with what we estimated to be the level of detail required in a standard NRES PIS. The third level was even more sophisticated and normally included links to academic articles or other non-lay sources directly related to the study, containing more information than on a standard PIS. An example of the second domain with the three folds is presented in appendix 1.

The readability of the PIS in each level was calculated using the Flesch–Kincaid reading ease score and grade level. The higher the reading ease score and the lower the grade level the easier it is to read and understand a document. Whether existing readability measurements can accurately evaluate the readability of provided health information is debatable.[17 18] Evidence suggests that the Flesch–Kincaid scale is widely used in studies of readability, has excellent repeatability and high correlation with other established readability scales (r=0.87–0.90).[19 20] In addition, Kim and colleagues[21] showed that readability scores between four different measurement scales, including the Flesch–Kincaid scale, were similar when compared with a health-specific readability measure that takes into account the text unit length alongside semantic and syntactic features of the text.

The information provided in all levels of the PIS had a mean reading ease score of 65.4 and a mean grade level score of 8.8, which indicates that the text was expected to be understood by an average student in the 8th grade (usually around ages 13–14 years according to the English educational system).[22] The readability statistics are displayed in table 1. We calculated the average time needed to read each domain of the information, based on 200 words per minute, which is the number of words an average person can read in a minute.[23 24]

At the end of the questionnaire, participants could opt to complete a further short questionnaire about the information they read in the PIS. Participants were asked to choose all options that applied to them from the following list: (1) I didn't click any of the (+) signs options; (2) I didn't find the information under (+) very useful; (3) I didn't find the information under (+) very interesting; (4) I found the information under (+) interesting but it didn't influence my decision to complete the questionnaire; (5) I would not have completed the questionnaire without being able to read the information under (+); (6) I would have liked more information about the project; (7) I would have liked more information about the questionnaire; (8) I would have liked more information about what you are going to do with the results of your study.

Table 1 Readability statistics for all levels of domains

PIS	Levels	No of words	Flesch–Kincaid reading ease score (grade level)
1. What is our research about	1	21	72.7 (8.3)
	2	84	54.5 (10.8)
	3	208 plus external link	34.9 (14.1)*
2. Why are we doing this research	1	28	42.4 (14.2)
	2	137	38.8 (14.6)
	3	328	42.2 (13.1)
3. Why have you been invited to take part	1	32	87.7 (5.2)
	2	160	54.4 (12.20)
	3	16 plus external link	–†
4. What would we like you to do	1	57	73.2 (7.7)
	2	82	75.3 (6.1)
	3	367	72.4 (8.2)
5. Who will see the information collected	1	30	56.2 (9.1)
	2	257	55.7 (10.8)
	3	150 plus two external links	63.9 (9.1)
6. What will happen to the information collected	1	43	60.4 (8.3)
	2	132	55.0 (11.0)
	3	13 plus external link	27.4 (14.6)

*Readability statistics are derived only from the text provided and not from the content in the links.
†Could not derive readability statistics on this level.

Statistical analysis

The baseline characteristics of the population were recorded. The number of the participants who entered each level of the domains, the actual time they spent reading this information, and the number of the participants who assessed the PIS were calculated. To explore whether there were any differences based on the participants' sex, socioeconomic status, ethnicity, age or the age of the twins and the information they accessed, we calculated the expected mean scores of the maximum level of information accessed and the time spent for every question by each category of the sample characteristics. All analyses were performed using the statistical software package STATA 11.

RESULTS

Of those who completed the survey and spent time reading the PIS, 98% (n=540) were women and 2% (n=12) were men. With regard to the educational level, 66% (n=309) of the participants had a university education, 20% (n=93) had a college/professional qualification and 14% (n=64) had a high school or lower education. Of those who participated, 98% (n=488) were white and 2% (n=10) of other ethnic background (Asian, black or mixed ethnicity). At the time of the survey, 55% of the participants (n=270) were employed and 45% (n=222) were not employed. With regard to the age of the sample, 8% (n=39) were between the ages of 20 and 30 years, 77% (n=360) were between 31 and 40 years old and 15% (n=68) were between the ages of 41 and 50 years (table 2).

Most participants (77%) chose to access the first level of information of each domain. Only 12% accessed the first and second level and 6% accessed all three levels of the domains. More specifically, 82% of the participants accessed the first level of the question on what participants will have to do, whereas only 11% accessed the first two levels and 7% accessed all three levels of the same question. The first level of the information on what the research was about was accessed by 80% of the participants, whereas 18% of the participants accessed the first two levels and 12% accessed all three levels. The rest of the questions follow the same pattern, with the first level being the more accessed (from 70% up to 76% of the participants) and the remaining the levels accessed by only a minority (from 3% up to 11% of the participants; table 3).

Table 2 Frequency distribution table of the main sample characteristics

	N=552	
Participant characteristics	**n**	**%**
Gender		
Female	540	98
Male	12	2.0
Education		
High school diploma or less	64	13.7
College/professional	93	20
University	309	66.3
Employment		
Employed/working	270	54.9
Unemployed/not working	222	45.1
Ethnicity		
White	488	98
Other	10	2.0
Age, years		
20–30	39	8.3
31–40	360	77.1
41–50	68	14.6

n may be less than N in any subtabulation due to missing data.

The actual time participants spent on each level of every domain is displayed in table 4. The estimated time needed to read the content is also presented. Generally, participants spent more time on the second and third levels of information. On average, the participants spent more time on information about why the survey was being done (25 s), on what participants were being asked to do (20 s) and on what the research was about (17 s).

Participants spent approximately 3 s less than the average reading time on the first level of information about 'what would we like participants to do', which was the most accessed information. They also spent less than the average reading time for levels 2 and 3 of this question. For the second more accessed information relating to 'what our research is about', the anticipated reading time was 6.3 s, whereas the participants spent 7.6 s (a difference of 1.3 s) more time reading the first level than the average reading time. They spent more time reading levels 2 and 3 but still less time than the average person would need to read and comprehend the content. By contrast, participants spent more than the average reading time on the information provided on the first level about 'why are we doing this research' (difference of 9 s).

There was no statistical difference in the pattern of accessing and time spent on the three levels of information between white and non-white ethnic groups, or a difference in the educational level of the parents and the age of the twins. Participants aged 41–50 years spent more time ($p=0.03$) reading the question on 'what is our research about?' than those in the other two age groups. Women were more likely than men to access at least the first level of information concerning 'what is our research about?' ($p<0.01$) and 'why are we doing this research?' ($p=0.02$). Men were more likely than women to spend more time on the information on 'why have you been invited to take part?' ($p<0.001$).

We also wanted to assess whether participants' perceptions about the quality of the information they read correlated with the actual time spent reading the information provided. The results on how participants perceived the information they read suggested that 34% (n=160) found the information interesting but it did not influence their decision to complete the questionnaire. Twenty per cent of the participants (n=93) would have liked more information about what we are going to do with the results of the study, even though only 6% clicked through to the third level of information. Seventeen per cent (n=82) said that they would not have completed the questionnaire without being able to read the information under the frequently asked questions, 15% (n=71) would have liked more information about the project, which again contrasts with the number who actually accessed higher levels of information (see table 3). Six per cent (n=30) would have liked more information about the questionnaire. Four per cent (n=20) said that they did not click any of the (+) sign options (which contrasts with the over 18% who we know did not click on any), whereas 3% (n=16) did not find the information interesting and 1% (n=3) did not find the information very useful.

DISCUSSION

As far as we are aware, this is the first empirical study to assess in detail the amount and type of information potential research participants use before they decide to participate in a research study. It recorded how much information was accessed and the actual time spent reading it was compared with the average reading times for the same text.

Table 3 Number/percentage of people who entered/clicked each level for every question

PIS Total n=552	Level 1 n (%)	Levels 1–2 n (%)	Levels 1–3 n (%)	Mean of levels 1–3 Mean (SD)
1. What is our research about?	446 (80)	78 (18)	53 (12)	1.29 (0.67)
2. Why are we doing this research?	425 (76)	50 (12)	27 (6)	1.18 (0.52)
3. Why have you been invited to take part?	419 (75)	41 (10)	23 (5)	1.15 (0.49)
4. What would we like you to do?	462 (82)	53 (11)	32 (7)	1.18 (0.54)
5. Who will see the information collected?	427 (76)	43 (10)	12 (3)	1.13 (0.41)
6. What will happen to the information collected?	390 (70)	35 (9)	22 (6)	1.14 (0.49)

Level 1 information was the most visited while information on levels 2 and 3 received much less attention. Few participants accessed levels 2 and 3 of information and spent little time looking at it, suggesting that the level of detail on standard PIS is not required by most participants in an online survey.

In the case of the most accessed domains in the PIS (information on what the research was about, what participants would have to do and why the survey was being done), the actual time reading and the average reading time for the first and second level were similar, suggesting that participants did read all the information accessed. When accessing the third level of the same domains, however, participants on average only spent approximately half (for the domains on what is our research about and why are we doing this survey) and approximately one-third (for the domain on what would we like participants to do) of the average reading time. This may have been because, having seen what was included, they found they were not interested in reading more detailed information; alternatively the level may have been accessed out of curiosity as to what lays behind the 'fold'.

Even though 20% of participants said that they would have liked more information about the study, only 6% accessed the third level of information and only 17% read the information that might be reproduced on a standard PIS (level 2). In short, even when there was information available it was not always utilised. Moreover, participants did not accurately report the extent to which they had actually accessed the information provided. More striking, perhaps, is the proportion of participants who were willing to take part without accessing any information in one or more domains before looking at the questionnaire. As table 3 indicates, between 28% and 30% (depending on the domain) chose not to access any information, and between 88% and 91% chose not to access information comparable to that provided in a standard PIS (level 2). When asked about the information provided, 34% stated that reading it did not influence their decision to complete the survey. We can speculate that rather than relying on the information provided, they went straight to the questionnaire and then decided on the basis of the kinds of questions being asked and the extent to which they found these intrusive, or on whether they felt that their answers would reveal anything they regarded as private or sensitive. We are unable to tell how many people chose not to participate after looking at either some of the information or the questionnaire itself as we only gathered information from those who chose to participate.

Nonetheless, the proportion of those who chose not to access information, or for whom it is reported not to have influenced decision-making, cannot be ignored for several reasons. First, it suggests that a significant minority of people did not want or use the information provided when they were actually making a decision about participation in the parent study. This requires further investigation, for example, to determine whether, taken together with the low uptake of information beyond level 1, too much weight is being placed on detailed PIS being available to questionnaire studies more generally. Second, taken together with the results on the reported use of information and the mismatch between information accessed and the reported need for more information, our results suggest at least a significant minority of participants actually rely less on the PIS to make a decision than can be inferred from the detailed scrutiny that these receive from research ethics committees. Third, the results highlight an ethical question about the responsibilities of researchers using online surveys. Should we have programmed the online system so that potential participants were unable to sign up to participate until they had spent at least the average reading time on all domains under level 2 (that which we regarded as being the standard PIS)? Of course, this would not have guaranteed that the information had been read, but it may

Table 4 Average stay time measured in seconds for each level and estimation of the average reading time needed per level per question (based on average adult reading 200 words per minute)

	Average time spent level 1 Mean (SD)	Average adult reading time level 1	Average time spent level 2 Mean (SD)	Average adult reading time level 2	Average time spent level 3 Mean (SD)	Average adult reading time level 3	Total time spent Mean (SD)
1. What is our research about?	7.6 (7.9)	6.3	24.0 (43.1)	25.2	43.7 (54.3)	62.4	17.4 (34.6)
2. Why are we doing this research?	17.3 (17.7)	8.4	29.0 (23.2)	41.1	50.7 (30.2)	98.4	24.6 (17.1)
3. Why have you been invited to take part?	7.3 (6.9)	9.6	14.3 (10.3)	48	19.3 (26.8)	External link*	10.0 (11.8)
4. What would we like you to do?	14.8 (74.9)	17.1	21.0 (44.2)	24.6	40.1 (19.9)	110.1	20.0 (77.2)
5. Who will see the information collected?	10.5 (66.5)	9.0	21.9 (21.9)	77.1	15.7 (12.9)	45	13.1 (66.7)
6. What will happen to the information collected?	11.9 (39.7)	12.9	17.0 (20.9)	39.6	16.6 (27.8)	Link to scientific paper*	14.5 (40.8)

The number of words on each level is displayed in table 1.
*The average adult reading time was calculated only for levels that included text and not links to external information.

be regarded as a safeguard for online studies in general. This, however, may raise many questions about what constitutes autonomous decision-making. The view that individuals can autonomously chose not to receive 'standard' information when making decisions is not without support, even in conservative bioethics.[23] Unfolding or otherwise interactive electronic information sheets undoubtedly permit potential participants to choose for themselves what information they need to make a decision. Refining them as a means of providing information will mean using them on studies in which the risks may be more significant. Taking seriously the idea that information needs vary from person to person means taking seriously the idea that some individuals may want to know less than we might ourselves, and that the duty to inform might be discharged by making a variety of information available rather than by insisting that everyone reads (or at least appears to have read) a fixed amount of information, with the tailoring only coming into play for those whose informational needs exceed this prescribed minimum. The extent to which research ethics committees will be comfortable embracing this as a principle in either research into participants' actual information needs or when applied to the more general use of tailored information (in which participants can actively choose to know less than may currently be required on a standard PIS) remains to be seen.

One challenge of this study was to determine whether our participants were actually using the information they accessed to inform their decision, given that they could click into a domain and have the browser open without actually reading the material provided. In the case of the first level of information, the comparison with average reading times is strongly suggestive that the materials were being read. In the case of the two further levels, things are less clear. The time spent on these domains was generally less than average reading times suggested were required to read them properly. On the other hand, potential participants may value the opportunity to skim read through the additional information, either picking out specific sentences of interest or to satisfy themselves that there was nothing further that concerned them. Accordingly, they may both value access to the information and consider that it does not influence their decision-making. Furthermore, the participants to this study knew that they could read through the questionnaire and then decide not to continue, which is easier in the case of internet-based studies than, for instance, personally administered paper questionnaires in which it might be harder to decide not to continue when the researcher is present. Internet studies are, however, similar in this regard to postal surveys, in which again, the paper version can be scanned before making a decision about whether or not to complete it.

There are limitations to the generalisability of the results of this study. Our participants were predominantly women (98%), white (98%), well educated (66% were university educated and 20% had college or professional qualifications) and all were under 50 years of age. The main study was an online-only study so our participants probably all had reasonably good computer skills and access to the internet. We were not able to record how many people decided not to participate nor, therefore, what information was accessed in order to make this decision.

CONCLUSION

Our aim was to examine the amount of information potential participants to the parent study read before they decided to participate, in order to inform discussions about how much information should be contained in a standard PIS. We were able to monitor in an innovative way what information the participants thought they would find most useful at the time a decision was required of them, and then how long they spent in each information domain. This time spent was then compared with average reading times to determine the likelihood that the participants had actually read all of the information on that domain, identifying that information was most significant for them to read based on how long they spent reading it. Level 1 information was the most accessed, ie, the briefest information, which was less than we would have anticipated being required for a standard PIS. Time spent on these areas was similar to the average reading times, suggesting that the information was actually read.

Our results on the participants' pattern of accessing and reading information suggested that the majority of our potential participants sought very little information before making a decision about whether or not to participate in our low risk, on-line, questionnaire-based study, and a significant minority felt they needed no information at all.

The NRES guidance for researchers and reviewers [3] has raised the concern that information sheets are becoming increasingly lengthy and complex, and may be deterring participation in clinical research. There is little evidence from which to determine how much information sheet participants actually need. A balance needs to be struck between overwhelming potential participants with too much information and giving them insufficient information to make an informed choice. Our study design offered a real possibility for personally tailored information, which may go some way to addressing this concern and improving participant understanding. It remains to be seen whether this method of tailoring information will be regarded as acceptable in clinical research.

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APPENDIX 1

1. What is our research about?
2. Why are we doing this research?
   - We would like to know whether the development of twins is influenced by the genes they inherit from their parents or by other environmental factors within the family.
   - Around two out of three sets of twins are non-identical (fraternal). In twin studies we assume that identical and non-identical twins do not differ in the way they are treated by their parents, thus they share a common family environment. Therefore, doing a twin study we can work out, by examining the differences between identical and non-identical twins, how much of a difference is caused by genetic factors and how much by environmental ones. In order to work out the genetic and environmental influences on trait variations and gather information on various aspects of your twins' behaviour we will ask you some questions on how your twins behave, how they think and understand what is happening, and how they react to in their surrounding environment. We will also ask you to answer some similar questions about yourself.
   - All the questions we will ask you have to do with the general growth and development of your twins. By analysing your answers to these questions, we will be able to work out whether your twins' development is influenced by the genes they inherit from you or by other environmental factors within the family. Also, in order to estimate these environmental factors, we need background information about the parents of twins. For example whether they smoke, how much they exercise now and before the twin pregnancy and their educational background. Specifically, we are interested in finding out how these factors, which describe the family environment, may influence twins' growth (their weight and height) and how they behave and feel. Also, by asking the parents to report which hand and foot they and their twins prefer, we will decide if the parents' preference of either the left or the right hand is associated with their twins' hand and foot preference. We need this information because we want to find out if there are any kind of differences between children who use their left hand and children who use their right hand. We already know that twins are more likely than singletons to be born with a low birth weight. Babies who are born prematurely or with a low birth weight may experience a greater number of neonatal complications and spend more time in hospital than other babies. Premature babies are more likely to have poor motor skills and poor abilities to adapt to new demanding situations, such as when they first start school, or meet new people. Several studies report that a delay in motor development is associated with low birth weight and shorter gestational age. Overall, prematurity and low birth weight are known to significantly affect the way a child develops. If we find out information about this, and information about your twins development we will be better able to pinpoint the role played by genetics and the environment.
3. Why have you been invited to take part?
4. What would we like you to do?
5. Who will see the information that is collected?
6. What will happen to the information that is collected?

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The Influence of Genetic and Environmental Factors on the Etiology of the Human Umbilical Cord: The East Flanders Prospective Twin Survey[1]

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ABSTRACT

The umbilical cord is vulnerable to a number of insults that may alter cord morphology, diminish cord flow, and ultimately compromise fetal nutrition. Thus, an investigation of the underlying mechanisms of the development of cord morphology and possible pathologies associated with it may provide insight regarding fetal growth in the intrauterine environment and have an impact on later development of the child. To our knowledge, this study, which included 11 980 twins, is the first to report the relative contribution of genes and environment in the development of the cord. Umbilical cord length, insertion, knots, twisting, and number of vessels were examined by trained midwives at birth. Means and percentages of cord characteristics by twin zygosity/chorionicity and gender were calculated. ANOVA and chi-square tests were performed to calculate discordance in cord morphology between dizygotic (DZ), monozygotic monochorionic (MZMC), and monozygotic dichorionic (MZDC) twins. Univariate genetic models were fit to the umbilical cord characteristics to investigate the genetic and environmental influences on umbilical cord morphology. Mainly nonshared environmental but also genetic factors influence umbilical cord morphology. In MZMC male and female twins, a peripheral/marginal cord insertion was significantly ($P < 0.01$) more prevalent compared to MZDC and DZ male and female twins, respectively. In MZMC male twins, clockwise twisting was significantly ($P = 0.02$) less frequent compared to DZ twins. Environmental and genetic factors influence cord morphology and pathology. Twin members can experience environmental influences that are not shared between them even in that very early stage of in utero life.

chorionicity, genetics, intrauterine environment, placenta, twins, umbilical cord, uterus

INTRODUCTION

The umbilical cord is the principal connection between the fetus and the placenta, providing the nutrients, oxygen, and fluids necessary for life in utero. The cord and its constituent tissues, an outer layer of amnion, porous Wharton jelly, two arteries, and one vein, are designed to provide and maintain the blood flow to the developing fetus [1].

Although the umbilical cord is one of the most vital components of the fetal anatomy, it is still one of the least-studied fetal structures. Given that the umbilical cord is vulnerable to a number of abnormalities that may occur during pregnancy, labor, or delivery, it is important to investigate the underlying mechanisms of the development of cord morphology and possible pathologies associated with it, because this may provide insight regarding fetal growth within the intrauterine environment and its impact on later life.

Some of the morphological aspects of the umbilical cord, such as its length, knots, insertion to the placenta, number of vessels, and twisting, have been associated with pathological outcomes [2–6]. At term, the typical umbilical cord length is 55–60 cm [4]. Adverse outcomes have been reported with both abnormally long cords (70–80 cm) and abnormally short cords (30–40 cm) [7]. Long or short cords can be the cause of hematomas and thrombosis of cord vessels and the surface of the placenta, thus causing fetal hypoxia, damage to the central nervous system, or even fetal death [8]. Infants with excessively long umbilical cords have a significantly higher likelihood of abnormalities on brain imaging and abnormal neurological follow-up in later life [9]. Results from a recent case report and literature review support earlier findings in which excessively long cords were associated with fetal loss, long-term neurological complications, and fetal thrombotic vasculopathy [10]. In a case-control study looking at infants with a diagnosis of short cords and infants without such a diagnosis, Krakowiak et al. [11] found that infants with short cords were more likely to have congenital malformations, be small for gestational age, and have more adverse outcomes, such as fetal distress and death within the first year of life. Moreover, it has been reported that decreased cord length correlates with depressed intelligence quotient values and psychomotor abnormality [12].

Less common but with potentially devastating consequences is the occurrence of cord knotting. In a population study of 69 000 singletons, knots were associated with grand multipar-

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ity, chronic hypertension, hydramnios, cord prolapse, and cord around the neck [13]. Moreover, a fourfold higher rate of antepartum fetal death was associated with these singletons. Airas and Heinonen [14] reported that knots were associated with previous miscarriages, obesity, long cord, and maternal anemia.

Variations in the site of the cord insertion to the fetus and the placenta and its relationship with later development have also been reported. The site of the cord insertion to the placenta is associated with later developmental abnormalities and growth restrictions [15–17]. These generally are categorized as placental insertions in the center, off the center, on the edge, or in the membranes. An insertion in the center of the placenta is more favorable compared to a marginal one that appears toward the edge of the placenta [18]. The marginal insertion, which is most commonly referred to as velamentous insertion, is the insertion into the membranes. This type of insertion is associated with the worst outcomes.

In a velamentous insertion, the veins traverse the membranes before they come together into the umbilical cord, and the cord inserts into the chorioamniotic membranes rather than on the placental mass. The incidence of this condition is 1.1% in singletons and 8.7% in twins. Moreover, the prevalence of velamentous cord insertion in monochorionic (MC) and dichorionic (DC) twin placentas is higher than in singleton placentas (12% and 7% vs. 2%, respectively) [19]. The presence of velamentous insertion is associated with an increased risk of birth weight discordance, especially in MC twins [20] and fetal vessel thrombi [21].

The intrauterine environment in which the twins develop has been suggested to have an important influence on later outcomes and is associated with the site and type of the insertion on the placenta. Unlike twins who have different chorions (DC twins), those who share the same chorion (MC twins) because of limited space may compete against each other for resources provided from the mother. MC twins are prone to adverse outcomes, which have often been attributed mainly to complications caused by placental vascular anastomoses [22, 23]. Very often, perinatal mortality rate is higher in MC twins than in DC twins [24–26], whereas the incidence of birth weight discordance is higher in MC twins [27].

Within the cord are two arteries and one vein—thus, three vessels in total. The two arteries send blood with waste products from the fetus to the placenta, and the umbilical vein sends oxygen and nutrient-enriched blood to the fetus from the placenta. Often, only two vessels are grossly visible: one artery and one vein. Single umbilical artery occurs in approximately 5% of cords in at least one twin, and it occurs more often in fetal demise than in live births [28]. Of infants with a single umbilical artery, 20% or more are reported to have associated fetal anomalies, including cardiovascular abnormalities [28] as well as a variety of renal defects and multiple anomaly syndromes [29, 30].

Counterclockwise/left or clockwise/right cord twists can be seen as early as the sixth week and are well established by the ninth week of development. Results from a case report by Herman et al. [31] suggested that large numbers of cord twists, causing torsion of the length of the cord, were found in two cases of intrauterine fetal death. Hypertwisted cords have also been associated with intrauterine growth restriction, nonreassuring fetal tracing, and increased rate of emergency cesarean section [6]. Obstetric outcomes and findings from placental examination have revealed that placenta previa, third-trimester bleeding, and single umbilical artery [32, 33] are more likely to occur in cases with a clockwise twist compared to those with a counterclockwise twist.

To the best of our knowledge, no study has so far investigated the relative contributions of genetic and environmental influences on the different characteristics of the umbilical cord. Specifically, we set out to examine the genetic and environmental etiology of cord length, insertion, knots, twisting, and number of vessels by employing a genetically sensitive design in a large population of twins.

MATERIALS AND METHODS

Sample

The East Flanders Prospective Twin Survey (EFPTS) has recorded multiple births in the province of East Flanders (Belgium) since 1964. From an initial sample of 6315 twin pairs registered with EFPTS from 1964 to 2002, a total of 205 twin pairs were excluded because of one or two twins being stillborn, and 120 twin pairs were excluded because of congenital malformations. This resulted in 5990 twin pairs, and a total of 11 980 twins, in the final analyses. Placentas were collected at birth and examined by a trained midwife within 48 h after delivery. Fetal membranes were carefully dissected as fully described by Derom and Derom [34]. All parents gave their written informed consent according to the local ethics committee guidelines. This project was approved by the Committee of Medical Ethics of the University of Leuven in Belgium.

Zygosity was determined by sequential sex, placentation, blood groups, and since 1985, DNA fingerprinting. Opposite-sex and same-sex twins with at least one different genetic marker were classified as dizygotic (DZ); MC twins were classified as monozygotic (MZ). For all the same-sex DC twins, a probability of monozygosity was calculated. After DNA fingerprinting, a probability of monozygosity of 0.999 was reached [35].

Measures

The umbilical cord was examined by a trained midwife at birth. In this analysis, cord length was treated as a continuous variable. The six categories of cord insertions were divided into two groups: central insertion (central, peripheral, and paramarginal) and peripheral insertion (marginal, membrane septum, and membrane peripheral). Because velamentous insertions have been previously associated with poor perinatal outcomes and quite often with fetal hemorrhage [36, 37], we repeated our analysis after combining the categories central, peripheral, paramarginal, and marginal into the nonvelamentous category and the categories membrane septum and membrane peripheral, which are cases of velamentous insertions on the cord, into the velamentous category. Cord knots were categorized as real knots, false knots, and no knots. Because knotting is more common in monoamniotic monochorionic (MoMo) twins [14], MoMo twins with cord knots were excluded from the analysis.

Cord twisting was categorized as clockwise, mixed/undefined, and counterclockwise. The number of cord vessels was categorized as two vessels and three vessels. The ordering of the knots and twisting was based on previous literature suggesting that more adverse outcomes are associated with real knots [38] or clockwise twisting [32, 33] whereas the frequency of problems diminish in the other categories.

Genetic Analysis

Intrapair twin correlations and pairwise concordance rates were calculated to examine the genetic and environmental influences on these phenotypes. The correlations of the categorical variables were estimated using the Spearman coefficient [39], and the correlations of the continuous variable cord length were determined using the Pearson coefficient [40]. Threshold models for cord knots, insertion, number of vessels, and twisting were used assuming an underlying liability scale adjusting to a threshold model of inheritance [41].

Variance decomposition was applied to the liability for cord knots, insertion, number of vessels, and twisting, leading to an estimate of the heritability of liability [42]. A maximum likelihood fit function was calculated based on raw data using the statistical software package Mx [43]. The estimates of the heritability of the liability are presented with 95% confidence intervals (CIs) and goodness-of-fit statistics for three models: 1) a full ACE model, in which the phenotypic variance is explained by genetic (A), common environmental (C), and nonshared environmental factors (E); 2) a model in which all variance was attributed to genetic and nonshared environmental factors (AE); and 3) a model in which all variance was explained by nonshared and common environmental factors (CE). Reduced models were estimated by removing one of the parameters at a time and rerunning the model. The

goodness of fit of the reduced models was compared to the full model to assess whether the reduced models represented a better explanation of the data using the likelihood ratio chi-square test and the Akaike information criterion. The models were assessed by examining the decrease in the fit of the model; if a parameter could be dropped without a significant decrease in fit, then on the grounds of parsimony, the reduced model was accepted as the best-fitting model [43].

Gender-Effect Genetic Models

The analysis was further extended to examine possible differences in the etiology of the variations in cord morphology between males and females. We fitted a general-effects (heterogeneity) gender-limitation model, a common-effects gender-limitation model, and a gender-homogeneity model [43]. By comparing a general heterogeneity model to a common homogeneity model, we investigated whether different genes influence the cord characteristics in males and females. Comparing the common-effects gender-limitation model to a homogeneity model, we tested whether any difference exists in the magnitude of the genetic influences on the etiology of the cord morphology in males and females.

RESULTS

Table 1 provides the mean cord length and the percentages for the remaining cord characteristics according to twins' gender, zygosity, and chorionicity. Cord length, knots, and vessels did not differ statistically between DZ, monozygotic dichorionic (MZDC), and monozygotic monochorionic (MZMC) male and female twin pairs. For both male and female twins, cord insertion differed statistically between DZ and MZMC and MZDC twins ($P < 0.01$), with more central insertions in DZ twins. In males, MZMC twins had significantly more peripheral insertions than MZDC twins ($P < 0.01$), whereas for females, the peripheral cord insertion was high in both MZMC and MZDC twins compared to DZ twins. A recategorization of the cord insertion into velamentous and nonvelamentous insertions (as described in *Materials and Methods*) led to results similar to those described above. DZ male and female twins had significantly ($P < 0.01$) more nonvelamentous insertions compared to the other groups. However, with this categorization, we found that MZDC male and female twins had more velamentous insertions ($P < 0.01$) compared to MZMC twins. In males, DZ twins had significantly more counterclockwise twisting than MZDC twins, and MZMC twins had significantly more counterclockwise twisting than MZDC twins ($P = 0.02$). Among female twins, cord twisting did not differ statistically.

The concordance rates for cord length were MZMC = 64%, MZDC = 56%, and DZ = 49%. For knots, the concordance rates were MZMC = 38%, MZDC = 31%, and DZ = 23%. For insertion, the rates were MZMC = 0%, MZDC = 20%, and DZ = 8%. For number of vessels, the rates were MZMC = 8%, MZDC = 24%, and DZ = 7%, and for twisting, they were MZMC = 9%, MZDC = 9%, and DZ = 8%.

The intrapair correlations for each cord characteristic according to twins' gender, zygosity, and chorionicity are presented in Table 2. For cord insertion, the lower intrapair correlation for female DZ twins ($r = 0.04$) compared to male DZ twins ($r = 0.12$) suggests a possible gender effect. The lower intrapair correlation in DZ females ($r = 0.16$) compared to DZ males ($r = 0.24$) suggests a possible gender effect for cord knots. For the rest of the cord characteristics, intrapair correlations do not suggest any significant differences between the groups regarding chorionicity or gender effect. Based on the finding that a gender effect may exist for cord knots and insertion, we proceeded with fitting gender-effect models to the data to further investigate the gender influence on cord knots and cord insertion heritability estimates.

Univariate Genetic Analyses

Variance estimates of ACE models and submodels and their 95% CIs are presented in Table 3, in which the best-fitting model is highlighted. The analysis for cord length suggested that genetic factors explained 30% (95% CI, 23%–37%) of the variance, a common environmental factor explained 32% (95% CI, 26%–38%) of the variance, and a nonshared environmental factor accounted for 38% (95% CI, 35%–40%) of the variance.

For cord knots, genetic factors accounted for 27% (95% CI, 26%–38%) of the variance, common environmental factors explained 23% of the variance (95% CI, 14%–31%), and nonshared environmental factors explained 50% of the variance (95% CI, 46%–53%). For cord insertion, 24% (95% CI, 18%–30%) of the variance was explained by common environmental factors, and nonshared environmental factors explained 76% of the variance (95% CI, 70%–82%).

For cord vessels, 41% (95% CI, 40%–57%) of the variance was explained by common environmental factors, and 59% (95% CI, 43%–77%) was explained by nonshared environmental factors. For cord twisting, 34% (95% CI, 29%–34%) of the variance was explained by common environmental factors, and 66% (95% CI, 66%–71%) of the variance was explained by nonshared environmental factors.

Gender-Effects Model

No significant difference was found between the general sex-limitation model and the common-effects gender-limitation model for cord insertion ($\Delta\chi^2 = 0.49$, df = 1) or for cord knots ($\Delta\chi^2 = 1.24$, df = 1). Additionally, no significant difference was found between the common-effects sex-limitation model and the homogeneity model ($\Delta\chi^2 = 6.61$, df = 4) for cord insertion or for cord knots ($\Delta\chi^2 = 3.79$, df = 4).

DISCUSSION

To our knowledge, the present study, which employed a twin research design with a sample of more than 10 000 twins, is the first to examine the relative contributions of genetic and environmental determinants to the characteristics of the umbilical cord. The heritability analyses suggested that genetic and unique intrauterine environmental factors play a substantial role in explaining the majority of the variation of the cord characteristics.

Heritability Analysis

The length of the cord is thought to reflect fetal movement in utero, often resulting in knotting [44]. Reduced cord length is associated with constraint in utero, which is more likely to evoke intrauterine problems, with the fetus moving randomly within a confined space during pregnancy. Both short and long cords have been associated with hematomas and thrombosis of the cord vessels and the surface of the placenta, which could lead to damage of the fetal central nervous system and sometimes even fetal death. Illustratively, cords from fetuses with Down syndrome, which is normally associated with hypotonicity and reduced fetal movement, tend to be shorter, with fewer coils, compared to those from healthy fetuses [44]. Genetic, common, and unique environmental factors seem to regulate the formulation of cord length and knots. It can be speculated that MZ twins are exposed to a more adverse fetal environment because of different placental circumstances, such as the sharing of one chorion and/or amniotic sacs [45], which can regulate the length of the cord and the knotting. A knot can constrict the blood vessels and lead to fetal death. If the knot is

TABLE 1. Means (SD)/frequencies of cord morphology by zygosity/chorionicity and gender.[a]

	DZ				MZDC				MZMC			
Cord morphology [b]	Male	n	Female	n	Male	n	Female	n	Male	n	Female	n
Length (cm)/SD	35.31 (11.46)	3041	33.85 (11.13)	2384	35.49 (11.38)	528	33.65 (10.24)	537	35.07 (10.85)	1209	33.87 (11.11)	1260
P value	0.74		0.92		0.74		0.92		0.74		0.92	
ANOVA	–		–		–		–		–		–	
Knots (%)												
Real	0.52	3063	0.31	2869	0	530	0.56	539	0.08	1222	0.24	1266
False	22		20.32		21.89		22.45		20.79		21.17	
No	77.47		79.39		78.11		76.9		79.13		78.59	
P value	0.10		0.61		0.10		0.61		0.10		0.61	
ANOVA	–		–		–		–		–		–	
Insertion (%)												
Central	90.71	3217	90.48	3035	77.43	545	70.65	552	71.26	1256	70.07	1293
Peripheral	9.29		9.52		22.57		29.35		28.74		29.93	
P value	<0.01		<0.01		<0.01		<0.01		<0.01		<0.01	
Chi-square	DZ>MZMC, MZDC; MZMC>MZDC		DZ>MZMC, MZDC		DZ>MZMC, MZDC; MZMC>MZDC		DZ>MZMC, MZDC		DZ>MZMC, MZDC; MZMC>MZDC		DZ>MZMC, MZDC	
Vessels (%)												
Two	1.13	3732	0.96	3524	1.69	533	1.47	545	1.70	1232	1.73	1271
Three	98.97		99.04		98.31		98.53		98.30		98.27	
P value	0.21		0.12		0.21		0.12		0.21		0.12	
Chi-square test	–		–		–		–		–		–	
Twisting (%)												
Clockwise	61	3033	62.35	2834	66.42	530	62.22	540	59.18	1215	60.47	1247
Mixed or undefined	26.41		24.35		21.32		24.44		26.91		25.58	
Counterclockwise	12.6		13.3		12.26		13.33		13.91		13.95	
P value	0.02		0.52		0.02		0.52		0.02		0.52	
Chi-square test	DZ>MZDC, MZMC>MZDC		–		DZ>MZDC, MZMC>MZDC		–		DZ>MZDC, MZMC>MZDC		–	

[a] SD, standard deviation; ANOVA, analysis of variance; n, number of participants.
[b] ANOVA/chi-square of cord features: difference between DZ, MZDC, MZMC.

TABLE 2. Intrapair twin correlations for each gender by chorion type and zygosity.[a]

	Male			Female			
Cord morphology	MZMC (n = 640)	MZDC (n = 276)	DZ (n = 2871)	MZMC (n = 657)	MZDC (n = 282)	DZ (n = 2765)	OS (n = 1882)
Length	0.62	0.56	0.50	0.67	0.58	0.52	0.49
Knots	0.34	0.34	0.24	0.42	0.27	0.16	0.26
Insertion	0.02	0.25	0.12	−0.03	0.16	0.04	0.08
Number of vessels	0.20	−0.01	−0.01	−0.02	0.57	0.10	0.09
Twisting	0.14	0.08	0.15	0.08	0.20	0.11	0.06

[a] OS, opposite sex; n, number of participants.

loose, the fetal circulation is maintained, albeit at a diminished rate. The tightening knot can occlude the circulation between the placenta and fetus and therefore obstruct the circulation of food supply, leading to compromised feeding [46]. One possible mechanism of the genetic effect on knots and length is the influence of a genetic predisposition on the amount of twin movement, which in turn impacts on these characteristics of the intrauterine environment.

For cord insertion and number of vessels, the heritability analysis suggested that environmental factors, both shared and individually unique environmental factors, explain most of the variance in cord characteristics. The site of the placental implantation is thought to be important: The upper part of the uterus is the most favorable area for placental implantation, because it is rich in blood and therefore nutrients and oxygen. The lower part of the uterus, however, is not; thus, more risks are associated with a low implantation [47]. However, certain conditions may predispose to a low implantation. Low implantations of placenta have a strong tendency to migrate upward toward the body and the base of the uterus, which can shift the cord toward a marginal or even velamentous cord insertion [48]. It is believed that the occurrence of marginal cords, with no signs of genetic predispositions, is specific to the vascular supply and peculiarities of an individual's uterus, including uterine scars, infections, or prior pregnancy complications.

Broadly, these results suggest that even in that early stage of life and regardless of the shared environment, individual twin members can uniquely experience situations within the same intrauterine environment. However, for cord insertion, chorionicity seems to be an important factor affecting the insertion (discussed below).

Our results suggest that nongenetic factors affect the twisting of the cord, with shared and nonshared environmental factors explaining most of the variation. Previous research has focused on whether twisting is genetically determined, inherent to the cord itself, or the result of external/extrinsic forces. Fletcher [49] talked about a mechanical rotation in which the cord twists as a result of fetal movement or rotation in early gestation. On the other hand, alternative theories have suggested that twisting is the result of factors inherent to the cord itself. Malpas and Symonds [3] have suggested that the helical structure of the cord results from genetic differences in the direction of the fibers in vessel walls and that a reciprocal action between the vessel walls and flow rate of the fetal blood result in the umbilical cord twist. However, in the present

TABLE 3. Univariate genetic model-fitting for umbilical cord presenting full and nested models.[a]

Threshold of cord morphology	A (95% CI)	C (95% CI)	E (95% CI)	$\Delta\chi^2$	df	*P* value	AIC
Length							
ACE*	0.30 (0.23–0.37)	0.32 (0.26–0.38)	0.38 (0.35–0.40)	–	–	–	–
AE	0.66 (0.64-0.68)	(0)	0.34 (0.32–0.36)	101.52	1	0.00	99.52
CE	(0)	0.52 (0.50–0.55)	0.47 (0.45–0.50)	61.46	1	0.00	59.46
E	(0)	(0)	1.00 (1.00–1.00)	1526.59	2	0.00	1522.59
Knots							
ACE*	0.27 (0.26–0.38)	0.23 (0.14-0.31)	0.50 (0.46–0.53)	–	–	–	–
AE	0.53 (0.50–0.56)	(0)	0.47 (0.43–0.50)	22.53	1	0.00	20.52
CE	(0)	0.42 (0.39-0.45)	0.58 (0.55–0.61)	22.67	1	0.00	20.67
E	(0)	(0)	1.00 (1.00–1.00)	463.62	2	0.00	459.62
Insertion							
ACE	0.00 (0.00–0.06)	0.24 (0.18-0.30)	0.76 (0.70–0.82)	–	–	–	–
AE	0.23 (0.22–0.29)	(0)	0.77 (0.70–0.84)	17.21	1	0.00	15.20
CE*	(0)	0.24 (0.18-0.30)	0.76 (0.70–0.82)	0.00	1	–	−2.00
E	(0)	(0)	1.00 (1.00–1.00)	0.00	2	0.00	59.11
Number of vessels							
ACE	0.11 (0.00–0.64)	0.33 (0.00-0.57)	0.56 (0.35–0.77)	–	–	–	–
AE	0.49 (0.27–0.67)	(0)	0.51 (0.33–0.73)	1.22	1	0.27	−0.78
CE*	(0)	0.41 (0.40-0.57)	0.59 (0.43–0.77)	0.11	1	0.74	−1.89
E	(0)	(0)	1.00 (1.00–1.00)	18.25	2	0.00	14.25
Twisting							
ACE	0.00 (0.00–0.06)	0.34 (0.32-0.37)	0.66 (0.66–0.71)	–	–	–	–
AE	0.39 (0.38–0.44)	(0)	0.61 (0.56–0.67)	21.78	1	0.00	19.78
CE*	(0)	0.34 (0.29-0.34)	0.66 (0.66–0.71)	0.00	1	–	−2.00
E	(0)	(0)	1.00 (1.00–1.00)	102.37	2	0.00	98.37

[a] A, Additive genetic; C, shared environment; E, nonshared environment; $\Delta\chi^2$, difference chi-square test; df, degrees of freedom; AIC, Akaike information criterion.
* Best fitting model.

study, the prevalence of cord twisting for MZ twins compared to DZ twins is not much higher, suggesting that there may not be a profound genetic influence on the etiology of twisting. On the contrary, unique factors not shared between the twin pair influence the development of twists. It has been previously suggested that fetal activity can explain cord twisting. Some suggest that an accentuation of the natural narrowing of the Wharton jelly toward the fetus permits excessive movement, whereas others conclude that fetal motion and twisting may cause a focal diminution of the Wharton jelly [8]. If an umbilical cord is twisted, it is more likely to tighten in locations of less resistance, such as an area low in Wharton jelly. A possible explanation for the unique environmental influences on cord twisting may be each twin's different experience of circumstances in utero, such as an area low in Wharton jelly, resulting in the twin's unique motor activity.

The role of the common environmental factors in influencing twisting, however, is not negligible (14% of the cord twisting variation). Often, genes express themselves through the environment. The first environment of the twins is the uterus, where the genes from the parents and the genes of each twin operate. However, in the intrauterine environment, the genetic influence is likely to be shared, which means that it will impact on both twins equally. The intrauterine shared environment may alter the way the twists are demonstrated and formed in the uterus, but the consequences may be unique to individual twin members.

Chorion and Gender Effects

The comparison between MC and DC twins revealed that cord length, knots, and number of vessels were not statistically different between MZMC, MZDC, and DZ twins. In MZMC male and female twins, a peripheral/marginal cord insertion was significantly more prevalent than in MZDC and DZ male and female twins, respectively. This finding is consistent with previous research [19], suggesting that marginal cord insertion in twin placentas, and especially in MC and DC twin placentas, is higher compared to that in singleton placentas. MC twins, who experience higher intrauterine constraint, may have compromised placental development, resulting in abnormal cord insertion into the placenta, thus increasing the risk of cord vessel rupture [48]. Sharing a chorion may influence the site of placental insertion and therefore produce competition for resources within the pairs. Peripheral cord insertion has been associated with low birth weight [15]; thus, it is reasonable to accept previous findings supporting the notion that significant discordance in fetal growth may result either from interfetal transfusion or placental insufficiency [27]. Unfortunately, further analyses using a genetically sensitive approach to examine the effect of the chorionicity type on cord insertion could not be performed because of the negative correlations between MZMC twins and MZDC and DZ twins. Additionally, when we repeated the analyses having recategorized cord insertion as velamentous and nonvelamentous, we found that MZDC twins had significantly more velamentous insertions compared to MZMC twins, suggesting that the difference we found between MC and DC twins should be further explored in future studies.

In MZMC male twins, clockwise/right twisting was significantly less prevalent compared to MZDC male twins. For female twins, cord twisting was not statistically significantly different between the three groups. Previous studies comparing right and left twisting have shown that right twisting is associated with more adverse obstetric outcomes [32, 33]. However, our results showed that right twisting is less common in MC twins compared to the other two groups, suggesting that MC placentas may not necessarily imply a qualitatively different environment for the development of twisting and its impact on later growth [15, 50]. It is possible that the development of cord length, number of vessels, and knots in MC twins results from a similar prenatal environment compared to DC twins.

Finally, the results of the gender-effect analysis on the etiology of cord insertion and knots suggested no gender-specific influences. Differences in the magnitude of the genetic influences were measured by comparing models in which variance components were separately estimated for men and women to models in which they were equated across genders. This analysis suggested no difference between the two models and therefore no difference in the magnitude of the gene expression in males and females.

In conclusion, to our knowledge, no previous study has demonstrated the genetic and environmental effects on the variations of umbilical cord pathology and morphology, and this is the first study to report that partly genetic and unique environmental factors influence a number of the morphological characteristics of the overall umbilical cord development. Twins, and the genetic influences on them, can affect their own intrauterine environment, and even in the very early stages of life, twins can experience unique environmental influences.

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