Twin Studies

beyond nature - nurture

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Outline [click title to enter]

Chapter 1:

Introduction:
Nature-Nurture and beyond

Back to outline

The common theme of the papers gathered in this thesis is that they are all 'twin studies'. The goal of the introduction of this thesis is to describe the context of twin studies. Its aim is to give a brief introduction into the methodology of twin studies, trying to emphasize the unique advantages of this kind of research without minimizing the limitations of the twin method.

1.1. Nature-Nurture: Genetics and Epidemiology

Genetics is the science concerned with heredity and variation of a trait within a (human) population (Klug and Cummings, 1997). It involves the study of cells, individuals, their offspring and the population in which the organism lives. In addition, molecular geneticists focus at the molecular basis underlying the variation. This field of genetic research is one of the major scientific accomplishments of the last decade: methods for gene discovery were (and are still being) refined, resulting in the first draft of the complete DNA sequences of the human genome (see Nature, no 6822, 15 feb 2001). Once all DNA sequences are mapped, then the question about the function of these genes arises: how do they work? This field of *functional genomics* will be in full expansion for the future decades (Fraser and Marcotte, 2004a; Fraser and Marcotte, 2004b; Luscombe et al., 2001; Steinmetz and Davis, 2004).

Epidemiology is the science that investigates health and disease in human populations. Its aim is to prevent illness (Bouter, 1991). Therefore, much of epidemiological research is directed at finding the etiological factors of the disease. Disease can be caused by environmental and/or genetic factors and/or by the interplay between those factors. *Genetic Epidemiology* examines such interactions between environmental and genetic factors in the causation of the disease. Recognition of the genetic and/or environmental basis of the disease provides direction for detection, prevention and treatment of the disease (Morton, 1992; Roberts, 1985; Steinmetz and Davis, 2004; van Os and Marcelis, 1998).

Genetic epidemiology starts with the question 'what are the causes of variation in a particular trait?' and in particular, 'are there any *genetic* factors causing differences between humans?' Other questions derived from this first question, are:

- If there are genetic effects on human differences, how important are they? In other words, what is the impact of environmental and/or genetic factors on a particular trait or disorder?

Back to outline

- If there are genetic and environmental effects on human differences, are they independent or do they influence each other? In other words, do they affect the trait or disorder as two unrelated causal factors or do they correlate or interact with each other?

1.1.1. Nature-Nurture: Genetically sensitive designs

Genetic epidemiology is focused at explaining the differences between individuals. The study of this variability is a relatively simple matter when the population consists of animals (such as mice or rats): the environment can be directly controlled and/or the genes can be controlled in breeding experiments. In a human population, genetic control is unethical and the same is true for a large number of environmental factors. And even if an environmental factor can be controlled, the experimental modification of that factor may be quite different from the occurrence of the same factor in the real, naturalistic setting (Neale and Cardon, 1992). Nevertheless, genetic epidemiological research is possible in a human population. A disease or disorder can be studied by an epidemiological analysis in a sample of genetically related individuals. Family studies, twin studies, adoption studies and molecular genetic studies are examples of such genetically sensitive epidemiological designs. Each design has its own aims and its own disadvantages, listed in table 1 (Marcelis, 2001; van Os and Marcelis, 1998).

Back to outline

Table 1: *Most common types of genetically sensitive epidemiological designs*

	Aims	Remarks
FAMILY STUDIES	1. Identification of familial aggregation of diseases 2. Study of familial syndromal overlap Identification of biological markers of genetic transmission (endophenotypes) 3. Study of interaction of familial with other biological/social factors	Main advantage of family studies is the possibility of selection of epidemiological, representative samples yielding generalisable results. Main disadvantage is the difficulty to separate genetic from environmental influences
TWIN STUDIES		
- traditional	1. MZ versus DZ similarity as a test of genetic aetiology 2. Quantification of contribution of genes and environment to phenotypic variance	MZ pairs may be treated more alike by their parents than DZ twin, which may contribute to the greater degree of phenotypic resemblance of MZ twins, and may inflate the associated heritability estimates.
- twins reared apart	Direct estimate of the magnitude of heritable effects	Furthermore, it has been suggested that MZ twins are more similar than DZ twins with regard to important risk factors for psychiatric disorders such as complications of birth and pregnancy, which may also lead to spuriously high heritability estimates. Traditional twin methodology does not take into account assortative mating (which would tend to lower heritability estimates), and non-additive gene effects and genotype-environment interactions (which would tend to inflate heritability estimates).
- twins reared apart vs twins reared together	Direct estimate of the magnitude of common rearing environment	
- discordant MZ twins	1. Identification of non-genetic contributors to disease	
ADOPTION STUDIES		
- adoptee study	Resemblance between biological affected parents and adopted-away offspring indicates shared heridity	Adoptive parents are in the main a biased sample, having been screened by adoption organizations for elevated social stability and absence of mental disorder.
- adoptee rearing environment study	Variation of expression of disease in high-risk adoptees as a function of adoptive rearing environment indicates genotype-environment interaction	Biological parents of adoptees are a similarly biased sample, because of high rates of mental disorder and low level of social stability.
- adoptee's family study	Resemblance between affected adoptees and biological parents indicates shared heridity	Selective placement by adoption organizations according to ethnic, religious, socio-economic status and other characteristics may introduce bias
- cross-fostering study	Resemblance between affected adoptees with normal biological parents and affected adoptive parents indicates effect of rearing environment	
MOLECULAR GENETIC STUDIES		
- linkage studies	Making a statement about the likelihood of close physical proximity of disease and marker alleles by comparing the frequency of variants of a marker polymorphism in affected and unaffected members in groups of closely related individuals	Linkage studies are heavily dependent on unknown parameters such as gene frequencies and penetrance. Newer linkage methods using sib-pairs partly avoid such problems. Linkage studies are suitable for detecting genes of major effect, whereas it is likely that psychiatric disorders in most families involve multiple genes of small effect.
- association studies	Making a statement about the likelihood of close physical proximity of disease and marker alleles on the basis of a comparison of the frequencies of variants of a marker polymorphism in groups of cases and controls	Association studies are simpler to carry out than linkage studies but are "near sighted": they can detect genes of small effect, but only those that are very close to the marker. They are also prone to confounding but newer methods avoid this by using within-family control groups for allelic association analyses.

Back to outline

This thesis concentrates on the traditional twin study, a design with monozygotic (MZ) and dizygotic (DZ) twins that permits an estimate of the relative impact of genetic and environmental factors.

Large worldwide twin registers exist from which twin pairs can be selected (see Table 2). Many are spin-offs from specific research projects, usually in psychology or medicine (Boomsma et al., 2002). For example, the East Flanders Prospective Twin Survey (EFPTS) (Derom et al., 2002; Loos et al., 1998), was started by Prof.Dr. Robert Derom after the observation of lower degrees of intra-uterine hypoxia in second-born twins (Derom, 1965). Twin registers differ from each other in several ways such as size, points of interest (e.g. the EFPTS is the only register determining the chorion type of the twins), but one of the major differences is the way in which they recruited the twin pairs. Some twin registers recruited twins based on direct information of multiple births, such as the EFPTS, or by applying computerized filters including sharing of date of birth, place of birth, family name. These population-based registers try to collect data on as many multiple births in a specific region or country as possible (e.g. the EFPTS registers almost all multiple births in the province of East Flanders, Belgium). Other twin registers, such as the Dutch Twin Register, recruit twins through advertising and media-campaigns. Although this way of data collection depends heavily on the motivation of the twins (or their parents), this strategy is shown to be very effective and possible effects of ascertainment bias[1] can be minimized by use of statistical methods (Boomsma et al., 2002).

A crucial point in twin research is the determination of the zygosity of the twins: Monozygotic (MZ), sharing all their genes or Dizygotic (DZ), sharing on average 50% of their genes. In the EFPTS the zygosity is based on sex, placental examination (within 48 hours after delivery), blood groups and if necessary on DNA-fingerprints. For more than 95% of the 6500 twin included in the EFPTS, the zygosity is known with a probability of least 95%. (Derom et al., 2001).

As already pointed out, a main part of the future will consist of identifying genes that play a role in (psycho) pathology. As genotyping is becoming relatively cheap and fast (DNA samples can be collected through buccal swabs which can be simply mailed by regular post), the main challenge lies in data collection and in finding large samples of participating subjects (Thompson, 2001). Therefore the Genoeutwin project has been initiated. This project

[1] Ascertainment bias: a systematic distortion in measuring the true frequency of a phenomenon, such as a trait or a disease, owing to the way in which the data are collected. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. Nat Rev Genet 2002; 3: 872-82.

Back to outline

combines data from 6 participating twin cohorts, resulting in a collection of more than 600.000 twin pairs including more than 30.000 DNA samples (Boomsma et al., 2002). The future of twin research lies in further maintaining and developing the twin registers but also in extending international cooperation between the twin registers.

Table 2: Large twin Registers in and outside of Europe
Based on: (Boomsma et al., 2002; Boomsma, 1998) and on Twin Research, Vol 5, Number 5 (october 2002).

Twin registers by country	*Number of twin pairs*	*Primary interest*
Belgium		
East Flanders Prospective Twin Survey	6500	Epidemiology, placentation, congenital anomalies, perinatal factors
Scandinavia		
Danish Twin Register	65000	Aging and age-related health, metabolic and cardiovascular disease
Finnish Twin Cohort	?? 48000	Health, personality and substance abuse
Norwegian Twin Register	40000	Mental health, obesity, asthma, allergies, health behaviours and perceptions, perinatal influences on health
National Institute of Public Health Twin Panel	7668	Physical and mental health, asthma, allergies, obesity and health-related behaviours
Swedish Twin Register	57405	Cancer, cardiovascular diseases, dementia, depression, substance use/abuse, cognition, personality, aging and common complex diseases
Swedish Young Male Twins Study	1783	Risk factors for metabolic and cardiovascular diseases, obesity and behavioural risk factor
Germany		
Berlin Twin Register	65000	Complex diseases, health-related QTLs, pharmacogenetics
German Obsvervational Study of Adult Twins and the Bielefield Longitudinal Study of Adult Twins	2509	Temperament and personality
Italy		
Italian Twin Register	120000	Aging, dementia, cardiovascular diseases, multiple sclerosis, celiac disease, diabetes, asthma, allergies, thyroid diseases and behavioural disorders
Register of Italian Athletes	4719	Human biology and development, sport and high-level performance
Twin Epidemiological Register of Rome	15500	Lifestyle, development and ageing
The Netherlands		
Nederlands Twin Register	30335	Development, behaviour, emotional problems, cognition, depression, addiction and cardiovascular risk factors
UK		
St Thomas' UK Adult Twin Register	10000	Cardiovascular, metabolic, musculoskeletal, dermatological and ophthalmological diseases
Twins' Early development Study	16810	Longitudinal assessment of verbal and non-verbal cognitive development and delay, language development and delay, childhood behaviour problems
Northern Region Multiple	1216	Effects of multiple pregnancy, obstetric and paediatric

Back to outline

Pregnancy Register		management and outcomes of pregnancy
Australia		
Australian Twin Register	27582	General resource for medical and scientific research
Western Australian Twin Register	4729	Asthma, allergy, ADHD, early speech and behaviour
China		
Chinese National Twin Programme	4576	Aetiologies of common diseases and health-related behaviour
Japan		
Osaka University Aged Twin Register	12000	Aging, dementia, physical diseases, lipids, cognition, lifestyle, life satisfaction and quality of life
South-Korea		
Korean Twin Register	154783	Complex human diseases and traits
Sri Lanka		
National Twin Register of Sri Lanka	20294	Multidisciplinary research and international collaborations
USA		
Mid-Atlantic Twin Register	23000	Behavioural and psychiatric disorders
NAS-NRC Twin Register of WWII Military Veteran Twins	15924	Somatic and psychiatric diseases, aging
Vietnam Era twin Register	7500	Veterans health, effects of combat, psychiatric disorders and substance abuse
California Twin Program	13096	Aetiology of disease and genetic markers
Southern California Twin Register	2600	Social and moral development, childhood behaviour problems, cognitive abilities
Minnesota Twin Register	5599	Individual differences

1.1.2 Summary

Genetic epidemiology is the overlap between genetics and epidemiology and focuses on interactions between environmental and genetic factors in the causation of the disease. Its goal is to detect, prevent and treat diseases and disorders. Genetically sensitive study designs such as family studies, adoption and twin studies are optimal tools investigating genetic and environmental risk factors. Twin studies have the unique opportunity to disentangle genetic influences from environmental influences. Large worldwide twin registers are available, such as the East Flanders Prospective Twin Survey, from which twins can be selected for research projects.

Back to outline

1.2. Nature-Nurture: Genes and environment

It all starts with the *phenotype*. The phenotype is the physical appearance of a trait or a disorder and can be measured in real life as well as in experimental conditions. It can be observed or measured by a questionnaire, test or interview. The phenotype is the result of two factors: the genes (nature) and the environment (nurture). Using a twin study, the relative contribution of these two factors to the measured trait (for example, general cognitive ability) can be estimated.

1.2.1. Nature: Genes

The factor that represents a unit of inheritance is called *a gene*. The site of a gene on a chromosome is known as the *locus* of that gene. *Alleles* are alternative forms of the gene that occupy the same locus on the chromosome. They are often represented by the letters A and a, or B and b. The simplest system for a locus consists of only two alleles (e.g. A and a), but there also may be a large number of alleles in a system. The *genotype* is the chromosomal set of alleles for an individual. At a single locus, with two alleles, the genotype may be represented by AA, or Aa or aa. If a multiple locus is considered, the genotype of an individual may be symbolized by AABB, AABb, AAbb, AaBB, AaBb, Aabb, aaBB, aaBb or aabb, in the case of two loci. *Homozygosity* refers to a state of identical alleles at corresponding loci on homologous chromosomes. In contrast, *heterozygosity* refers to a state of unlike alleles at corresponding loci on the chromosomes.

Geneticists typically distinguish between two kinds of genetic effects: additive and non-additive genetic effects (Neale and Cardon, 1992). If the effects of different genes add together in their effect on the trait or disorder, this is called *additive genetic effect*. The *non-additive genetic effects* are the genetic effects that do not add together in their effect on the trait or disorder. Two types of genetic non-additivity are dominance and epistasis. *Dominance* represents the genetic effect of a combination of alleles present at a given chromosomal locus. *Epistasis* is said to occur whenever the effect of a gene depends on which genotype is expressed at another locus. While dominance describes the interaction between alleles at the same locus, epistasis describes the interaction between alleles at different loci. Because of the reassortment of genes during the human reproductive process, the effects of epistasis do not get transmitted from parent to offspring. Experimental studies in animals have shown a rich variety of possible epistatic interactions depending on the number and effects of the interacting loci (Andersson and Georges, 2004; Montooth et al., 2003; Williams et al., 2004).

Back to outline

However, in human studies it is almost impossible to disentangle epistatic effects because the specific loci involved in the epistatic effect are not yet identified (Moore, 2003; Purcell and Sham, 2004). When looking at the outcomes of twin studies or other genetic studies, it should be realized that those epistatic effects are not taken into account, which can lead to impure heritability estimates.

Heritability is the proportion of individual differences in a particular population at a given time that are due to genetic differences among individuals. It refers to the genetic contribution to individual differences and not to the genetic contribution to the phenotype of a single individual (Neale and Cardon, 1992; Plomin et al., 2000). The concept of heritability describes the contribution of genetic factors to observed differences among subjects in a particular population, at a particular time. The heritability estimate is in fact a population- and time-specific estimate. This means that it is important to find out if that estimate is stable over time (e.g. is the role of genes in a particular trait in childhood as important as in adulthood?), stable over populations (e.g. is the heritability estimate the same for both sexes?) and applicable to a general population (e.g. is the heritability estimate derived from twin studies the same as for a non-twin population?). When a disease or disorder is found to be heritable for a large part, this does not imply some kind of deterministic idea. Genes represent only genetic risk factors: they do increase the probability of occurrence of a disorder, but they do not guarantee that the disease or disorder will occur. In fact, something such as 'bad genes' does not exist. Genes are not good or bad, but are in most cases both, e.g. a gene (DRD4 dopamine receptor) associated with novelty seeking (Keltikangas-Jarvinen et al., 2003; Keltikangas-Jarvinen et al., 2004) may be a genetic risk factor for antisocial behaviour (Goldman et al., 1996), but it could also predispose to creativity (Plomin et al., 2000).

To avoid misunderstanding in science, heritability is defined in a broad sense as well as in a narrow sense. Heritability in a narrow sense takes only the additive genetic effects into account whereas heritability in a broad sense takes the additive as well as the non-additive (dominance) genetic effect into account. Most twin studies focus on the heritability of a trait in the narrow sense. They give an indication of the extent to which a particular trait will 'breed true', that is the degree to which offspring will resemble their parents (Plomin et al., 2000).

Back to outline

1.2.2. Nurture: The environment

Environmental factors are simply all influences other than inherited factors (Plomin et al., 2000). This concept is broader than usually defined in psychology: it refers not only to family socialization factors, but also to parental events and non-genetic biological events after birth such as illness and nutrition.

Two kinds of environmental influences are possible: 'non-shared environmental factors', and 'shared environmental factors'.

The shared environmental factors, also called '*the common environment'* or the *'family environment'* are all those environmental factors that are shared by twin pair members and therefore, tend to make twin pair members more similar to each other. Examples of shared environmental factors are going to the same school, living in the same household with its specific features or sharing the same hobbies.

The non-shared environmental factors, also called '*unique environment'*, '*individual-specific environment'* or '*random environment'* are all those environmental factors that are not shared between members of a twin pair. Examples are having different friends, having different interests, experiencing an individual-specific life event such as an accident.

Each scientific study has, by definition, some 'noise' included, meaning that there is some deviation from the reality. This is inevitable because no measurement instrument exists that fully and correctly covers reality. 'Noise' can also be caused by the statistics that are applied to the data. That part of the variation in a trait or disorder that is caused by 'noise' is also captured in the 'non-shared environmental factors'.

The strength of a twin study is that it can distinguish between the shared and the non-shared environment. A weakness is, however, that the non-shared environmental effects cannot be divided into that part that is caused by environmental effects and that part that is caused by 'noise'. In addition, twin studies cannot discriminate between the various sources of the shared environment. Other studies are needed to identify the specific environmental factors influencing the trait or disorder.

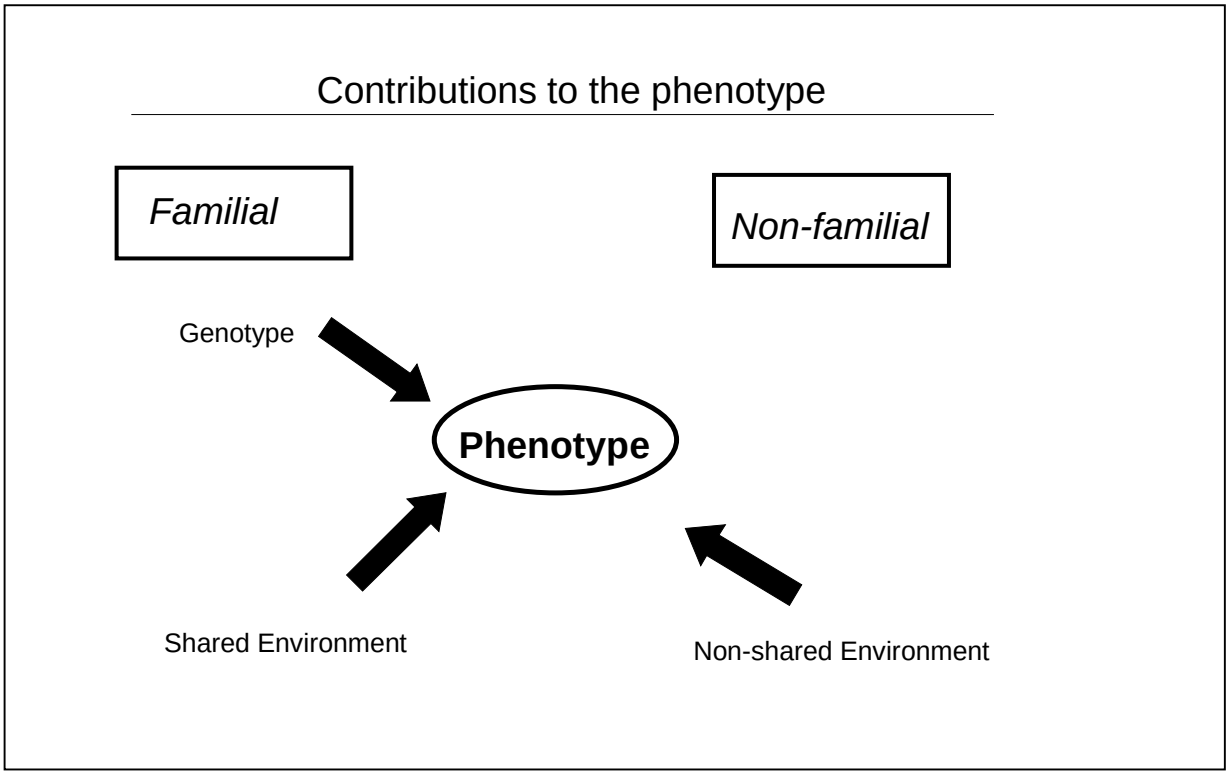
1.2.3. Summary

The phenotype is influenced by genetic factors, shared environmental and non-shared environmental. As genetic factors and shared environmental factors tend to make subjects belonging to the same family, more similar to each other and more different from other families, these are called 'familial factors' influencing the phenotype. Non-shared

Back to outline

environmental factors tend to create differences between members of the same family and are therefore called ‘non-familial factors’ influencing the phenotype.

Figure 1: *Contributions to the phenotype*



1.3. Modeling Nature-Nurture: the classical twin methodology

1.3.1. Heritability in narrow sense

The aim of a twin study is to estimate the relative contribution of genetic and (shared or non-shared) environmental factors. The intra-pair MZ and DZ correlations give an initial idea about the role of genes and/or environment. The basic idea is that if the intra-pair correlation of the DZ twin pairs is about half of that of the MZ twin pairs, genetic factors may play a role in the trait or disorder. After all, DZ twins share half of their genes and MZ twins are genetically identical.

Back to outline

The pattern of the intra-pair correlations is used as a guideline in the Structural Equation modeling process, the goal of which is to find the best fitting model (Cudeck et al., 2001; Kline, 1998). This process always starts with the "full" model.

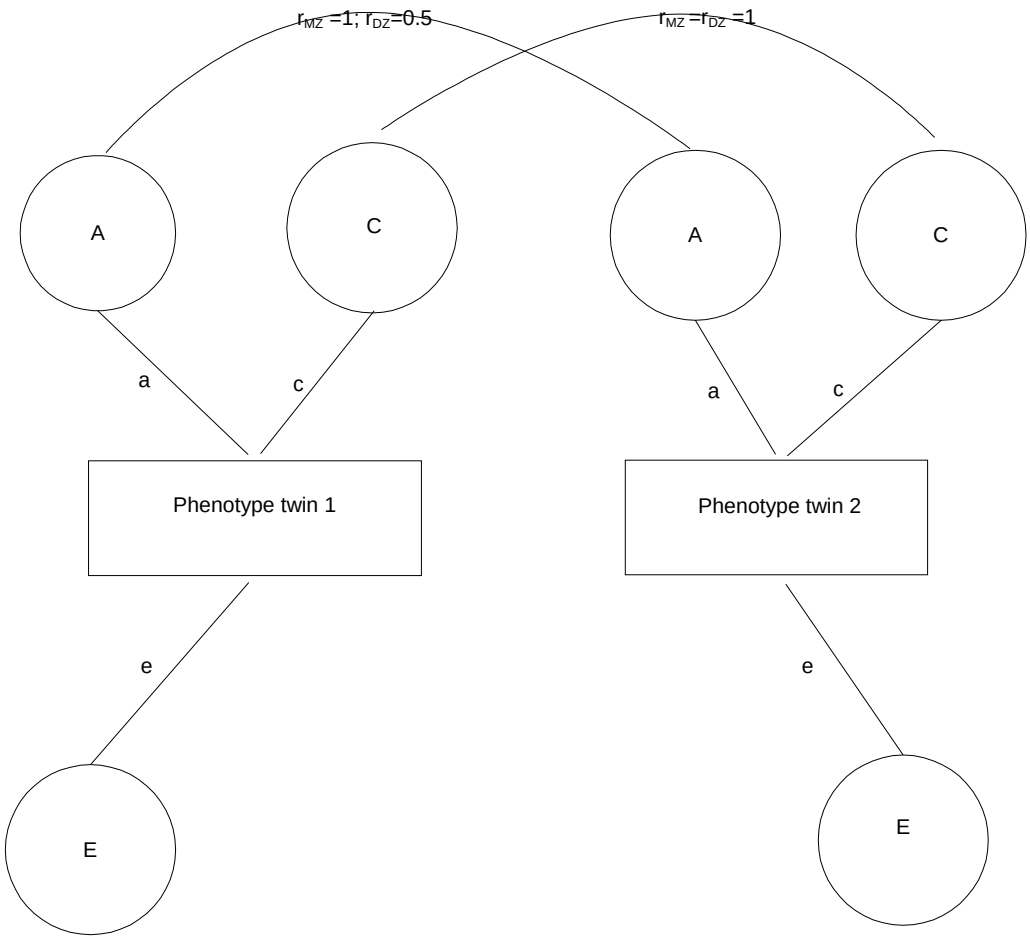


Figure 2: The ACE twin model

As figure 2 shows, the phenotype is influenced by three latent factors: (1) additive genetic factors (A), (2) shared environmental factors (C) and (3) non-shared environmental factors (E). As MZ twins are genetically identical, the correlation (r) between the additive genetic factors of twin 1 and twin 2 is fixed at 1. As DZ twins share half of their genes, the correlation (r) between the additive genetic factors is fixed at 0.5. The correlation between the shared environmental factors is fixed at 1 for both MZ and DZ twins, as long as they are reared together. The non-shared environmental factors are non-shared between members of a twin pair, so the correlation of those factors is fixed at 0. The size of each factor is represented by the path coefficient. This model is called the full ACE twin model. Based on the hypothesis

Back to outline

deduced from the correlation analysis, several models can be fitted to the data, e.g. a model with a non-shared environmental factor only and a genetic factor (a so-called AE model) or a model without a genetic factor (a so-called CE model). The models can be compared using several statistical criteria. The best-fitting model is chosen, based on likelihood (best fit) and parsimony of the model (highest degrees of freedom).

1.3.2. Structural Equation Modeling: Mx

There are several statistical packages available for structural equation modeling such as Mplus (Muthen and Muthen, 1998-2004), LISREL (Joreskog and Sorbom, 1986), LISCOMP (Muthen, 1988), EQ (Bentler, 1995), SEPATH (Steiger, 1995) and AMOS (Arbuckle, 1996) . Most twin researchers are working with the programme Mx, designed by Prof.Dr.M Neale (Neale, 1999). This package has some distinct advantages. First, it is free of charge. It can be downloaded from http://www.vcu.edu/mx/ . Second, it is developed with behaviour quantitative models in mind (such as the classical twin model). Third and perhaps the most important feature, it is not very difficult to apply. Models can be fitted through a user-friendly graphical interface or through scripts that can be written by the user itself. They can also be downloaded and then transformed according to one's own desires.
All twin data analyses described in the present thesis were carried out using Mx. The scripts used for the analyses are enclosed (see appendix B).

1.3.3. Equal Environment assumption: Perfect Nurture?

The validity of the classical twin method depends on several assumptions. One of them is the equal environment assumption (EEA), i.e. the assumption that MZ and DZ twins are equally correlated in their exposure to environmental factors of etiological importance for the trait that is being studied (Kendler et al., 1994).
A possible violation of the Equal Environment Assumption can arise if MZ twins are treated more similarly than DZ twins (e.g. going to the same class, wearing the same clothes, parents treating them more alike). This violation would result in an over-estimation of the heritability of the observed trait. However, studies investigating whether environmental similarity causes increased phenotypic concordance, showed that environmental similarity during childhood did not predict twin similarity in personality, attitudes, cognitive abilities nor in a range of psychiatric disorders (Evans and Martin, 2000; Kendler et al., 1994). Furthermore, if trait resemblance between twins is influenced by the similarity in which they are treated, then trait resemblance would be correlated with physical similarity, after controlling for zygosity

Back to outline

(Evans and Martin, 2000). Studies have shown that physical similarity is not correlated with similarity in personality, cognitive abilities or schizophrenia (Kendler, 1983; Matheny et al., 1976; Plomin et al., 1976).

Another way to test the EEA is to study the effect of labelling a twin pair as MZ or DZ in a sample of twins misclassified by their parents or by themselves (Kendler et al., 1993; Scarr and Carter-Saltzman, 1979). After all, if there is some preconceived notion that MZ twins are more alike than DZ twins and therefore should be treated more similarly, then trait similarity should be a function of the perceived zygosity (Evans and Martin, 2000). Studies have shown that this is not the case for intelligence, attitudes, hyperactivity and for psychiatric disorders such as depression, generalized anxiety disorder, phobia, alcohol abuse and bulimia (Kendler et al., 1993).

To conclude, the equal environment assumption has been tested in several ways and in different samples. Most studies have shown support in favour of this assumption.

1.3.4. MZ twins – genetically identical: Perfect Nature?

The basic assumption of the twin method is that MZ twins are perfect clones of each other. They are supposed to be 100% genetically identical. However, there are some reasons why MZ twins may be less than fully identical (Evans and Martin, 2000; Martin et al., 1997).

First, there exist two types of MZ twins: MZ-monochorionic (MZ-MC) and MZ-dichorionic (MZ-DC). About 65% of all MZ twins are monochorionic, meaning that they share the same chorion. However, the sharing of a chorion may make the twins more similar, or in some cases, more different, than twins that do not share a chorion (Prescott et al., 1999). If monochorionicity should result in MZ-MC having different intrapair correlations than the MZ-DC, then this would undermine the classical twin model, leading to an impure heritability. There are some studies focusing at the influence of chorion type on heritability estimates and they concluded that the influence of chorion type was minimal and negligible (Fagard et al., 2003; Loos et al., 2001a; Loos et al., 2001b; Prescott et al., 1999; Reed et al., 2002; Wichers et al., 2002) . However, it is always a good idea to investigate chorion effects on the phenotype, if data on chorionicity is available.

Second, there are also some biological processes such as unequal allocation of blastomeres, uneven cytoplastic distribution of DNA methylases, postzygotic nondisjunction and skewed X-inactivation in female pairs, that may contribute to differences between MZ twins (Evans and Martin, 2000; Martin et al., 1997). However, this is not prevalent and to distort the

Back to outline

classical twin method there must be a causal link between those DNA mutations and the phenotype that is being studied. In most cases, such a link is unlikely.

1.3.5. Twins representative of singletons from the general population?

Perhaps the most relevant question about the validity and reliability of the twin method concerns the generalisation of results coming from twin studies. Are twins really representative of singletons from the general population? It is well known that twins differ from singletons in several ways. They are delivered after a shorter gestation time (37 vs 40 weeks) and with a lower birth weight (Blickstein, 2004; Buckler and Green, 2004; Loos et al., 1998). This could make twins more at risk for developing diseases, especially cardiovascular diseases (Barker, 1999; Barker et al., 2002; Godfrey and Barker, 2001). The twin birth is also associated with greater risk of perinatal complications, resulting in increased risk for developing (psycho) pathology and increased mortality rates (Baird et al., 1998; Blickstein and Keith, 2004; Kurdi et al., 2004).

There are some studies reporting differences between twins and singletons, especially in the field of cognitive abilities (Posthuma et al., 2000). However, these studies focused on young twins, studies examining older twins did not replicate the differences between twins and singletons (Evans and Martin, 2000; Posthuma et al., 2000). It is now well accepted that, if there is any difference between twins and singletons, this difference would fade away at the age of five (Evans and Martin, 2000). However, it is always a good idea to test the representativeness of the twin sample, if possible. A good way to do this is to include singletons matched in genetic background and (early) environmental experiences, such as siblings in the study design (Evans and Martin, 2000). When twins do not differ from the siblings in terms of means and frequencies of the observed traits, then there is no reason to question the representativeness of the twin sample. If no sibling data is available, then the means and frequencies of the traits observed in the twin sample can be compared with data coming from the general population. It has been shown that twins and singletons from the general population did not differ in most traits and (adult) psychiatric diseases like schizophrenia or affective disorder (Chitkara et al., 1988).

1.3.6 Summary

The classical twin methodology consists of fitting different nested models to the data (structural equation modeling). The best-fitting model is chosen based on fit and parsimony, allowing estimating the impact of genetic and environmental factors on the observed

Back to outline

phenotype. Underlying assumptions of the classical twin models are i) the equal environment assumption, ii) the perfect genetic similarity between members of MZ pairs and iii) the representativeness of twins for singletons. Studies investigating these assumptions showed that, in most cases, the assumptions are valid. Twin studies are therefore a reliable method to disentangle genetic and environmental influences on an observed phenotype.

1.4. Beyond Nature-Nurture

The twin model, as described above, considers genes and environment as independent causal factors. It is assumed that genetic and environmental factors influence the phenotype independent from each other. However, the assumption that a trait is influenced by genetic and environmental factors separately is a simplification of reality. It has been realized that there can be interplay among the two: they can correlate or interact. Twin studies are now focusing on these genotype-environment effects.

1.4.1. Genotype-Environment Correlation

As the term suggests, genotype-environment correlation refers to the idea that the environments which individuals experience may not be a random sample of the whole range of environments, but may be caused by, or correlate with, their genetic makeup.

Back to outline

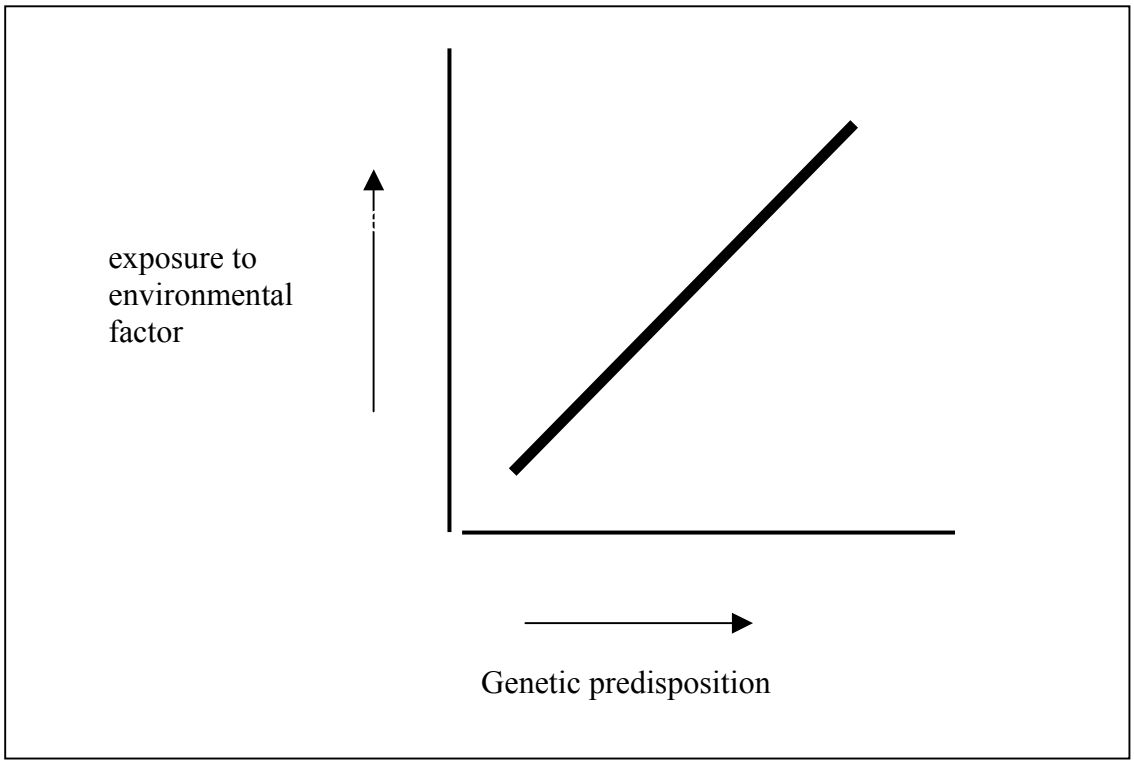


Figure 3: Genotype-environment correlation
(Marcelis, 2001; Neale and Cardon, 1992; Van Praag et al., 2004)

Three kinds of genotype-environment correlation can be distinguished: passive, active and reactive.

The first type (*passive genotype-environment correlation*) arises because the environment in which individuals grow up is often provided by their biological relatives. Thus, one's environment is provided by the phenotype of someone who is genetically related. Intellectually gifted persons may also be born and grow up into homes that provide them with more enriched and stimulating environments, having more money to spend on books and education and being more committed to learning and teaching. Another example, a child who inherits the genes that make him vulnerable to depression may be more likely to experience the pathogenic environment of rejection and neglect, because the tendency of parents to reject and neglect their children may be caused by the same genes that predispose to depression. In this case, a high genetic predisposition to depression is correlated with exposure to an adverse environment, because both genes and environment derive originally from the parents.

The second type (*active genotype-environment correlation)* arises because the individual creates, or chooses those environments that are functions of his genotype. For example, an intellectual gifted person would look for those environments that challenge his intellectual

Back to outline

capacity and would invest more time in mentally stimulating activities. A person with a genetic vulnerability for depression would ‘choose’ those ‘depressive environments’ that make him even more depressive.
The third type (*reactive genotype-environment correlation*) is based on the notion that individuals are reacted to on the basis of their genetic make-up. Intellectual gifted children might be picked out at school and given special opportunities. Subjects with liability genes for depression may elicit reactions from other people that confirm their own feelings of worthlessness and insecurity, increasing the risk to actually develop depressive complaints.

A large female twin study (Kendler, 1997) provided evidence for genotype-environment correlation in depression. It was shown that genetic liability to depression (defined as having an affected co-twin) was associated with a significantly higher risk experiencing stressful life events such as assault, marital problems, divorce/break-up, loss of job, serious illness, major financial problems and trouble getting along with friends and family, and this risk was more higher in MZ twins than in DZ twins. Thus, the risk of being exposed to stressful life events rises with an increasing genetic liability for depression.

1.4.2 Genotype-Environment Interaction

In the case of genotype-environment interaction, people do not experience different environments as a consequence of their genetic predisposition, as is the case with genotype-environment correlation. They experience the same environments but react differently according to their genetic predisposition.

Back to outline

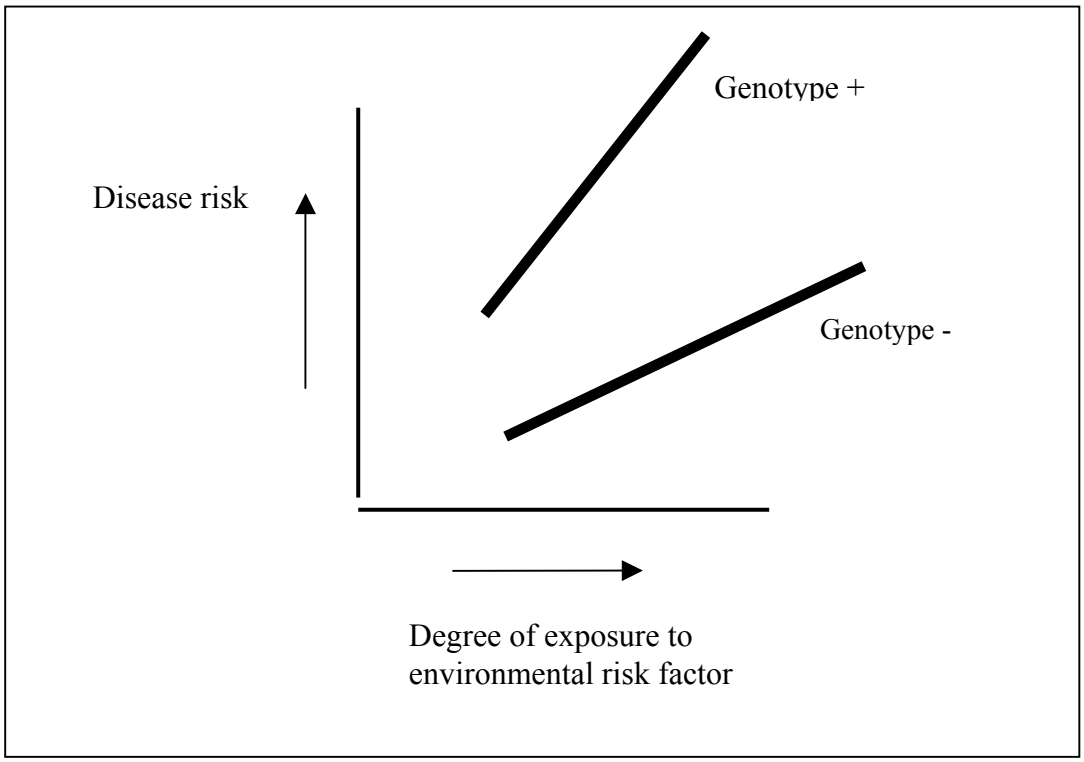


Figure 4: Genotype-environment interaction

(Marcelis, 2001; Neale and Cardon, 1992; Van Praag et al., 2004)

Another large female twin study by Kendler et al (Kendler et al., 1995) provided evidence for genotype-environment interaction in depression. It was shown that the risk of onset of major depression was increased after experiencing a stressful life event, and that this increase in risk was higher in MZ twins (with an affected co-twin) than in DZ twins (with an affected co-twin). Thus, the risk of developing depressive symptoms after exposure to a stressful life-event increases with higher genetic liability to depression. Liability genes for depression make (female) subjects more sensitive for the depression-inducing effects of stressful life events. Caspi (Caspi et al., 2003) confirmed this by actually identifying a functional polymorphism that moderated the influence of stressful life events on depression. This polymorphism was found in the promoter region of the serotonin transporter gene.

1.4.3. Summary

The classical twin model assumes that genetic and environmental factors act as two separate and independent causal factors, but it has been shown that this is simplification of what really occurs. Genes and environment can (passive, active or reactive) correlate or they can interact.

Back to outline

However, it has been shown that a twin design is also an optimal way to examine such genotype-environment effects.

1.5. Conclusion: Nature-Nurture and beyond....

Twin studies are a valuable tool in genetic epidemiology. They make the distinction between genetic and environmental influences on the phenotype and estimate the size of these influences.
However, it has been realized that genes and environment are not two separate and independent causal factors, but they can correlate and interact. Twin studies are now starting to focus on such effects.

It is remarkable that the enormous efforts made in the last two decades to identify genes associated with phenotypes such as general cognitive ability and psychopathology, have produced so little convincing results. All the hard work has not led to strong associations between genes and phenotypes. An explanation can be that most phenotypes are complex, meaning that it is unlikely that they are associated with one gene with a large effect but they are more likely associated with a lot of genes with small effects. Furthermore, molecular genetic studies mainly focus at additive genetic effects. As it has been shown that non-additive effects such as epistasis are also important in explaining the phenotype, this point of view can be too narrow to find associated genes. Even more, as twin studies are now showing that genes do not operate apart from the environment in which the individual lives, one can even raise the question about the usefulness of trying to find associated genes without taking the environment into account.

In fact, it can be concluded that both twin studies, which were originally designed to estimate genetic influences on the phenotype, and molecular genetic studies, which are focused at identifying genes associated with the phenotype, had an effect on science that was not expected. Instead of emphasizing the role of genetic factors alone, they both led to the conclusion that the environment is also important. Environmental influences on the phenotype cannot be ignored. Therefore, the future of twin studies as well as molecular genetic studies lies in including these environmental influences.

Back to outline

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Back to outline

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Back to outline

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Back to outline

Chapter 2:

Twin studies of this thesis:
Outline and aims

Back to outline

The papers in this thesis are related to two projects.

2.1. A twin study on cognitive ability

2.2. A twin study on stress reactivity in the flow of daily life

2.1. A twin study on cognitive ability

The first project was requested (and partly financed) by the Marguerite-Marie Delacroix foundation, and was aimed at examining general cognitive ability, and in particular cognitive disability, in a large sample of twins. Previous twin studies showed that genetic factors played an important role in explaining individual differences in cognitive ability (Boomsma, 1993; Plomin, 1999; Wright et al., 2001). However, none of these studies looked at the possible confounding effect of chorion type on the heritability estimates. As described in the previous chapter, ignoring the fact that about two thirds of the MZ twins share the same chorion, may lead to inaccurate heritability estimates. This study, therefore, investigated the effect of chorionicity on measures of cognitive ability. Six hundred and fifty young twins of the East Flanders Prospective Twin Study were assessed with the Wechsler Intelligence Scale for Children-revised (WISC-R). This IQ-test examines several cognitive abilities (such as specific verbal and performance abilities) and results in a measure of general cognitive ability (total IQ). Using structural equation modeling (see chapter one), the effect of chorion type on several specific cognitive abilities as well as on general cognitive ability was examined. Results of these analyses are described in chapter 3 of this thesis.

In addition, previous work demonstrated associations between lower cognitive ability and childhood non-psychotic psychopathology. This association has been found to be stable over time (Dietz et al., 1997; Goodman, 1995; Kusche et al., 1993) and has been reported in clinical as well in non-clinical samples (Cook et al., 1994; Hollaender and Hebborn Brass, 1989; Loney et al., 1998; Manikam et al., 1995). Furthermore, it is well established that both lower cognitive ability and childhood psychopathology are influenced by genetic and environmental factors (Devlin et al., 1997; Edelbrock et al., 1995; Plomin, 1999; van der Valk et al., 1998). Based on these findings, it was hypothesized that the association between lower cognitive ability and childhood psychopathology was caused by the same genes and/or environments. To investigate this hypothesis, the IQ data was combined with the data on behaviour gathered in the same twins, and bivariate structural equation modeling was applied. Results of these analyses are described in chapter 4 of this thesis.

Back to outline

2.2. A twin study on stress sensitivity in the flow of daily life

The second project, partly financed by Zon-Mw (grant number: 904-57-116), was a longitudinal twin study on stress sensitivity. Stress has become inevitable in our Western society. According to the WHO, stress is expected to be one of the major causes of dysfunction for the near future (WHO, 2001). Earlier research mainly examined the impact of major stressful life events on general health, such as difficulties in love relationships, serious illness and job loss, providing abundant evidence for a causal relationship (Lopes et al., 2003; Paykel, 2003). However, recently, it has been suggested that daily stressful occurrences might be even more important to general well being (DeLongis et al., 1988; Stone et al., 1993; van Eck et al., 1998). Although their impact may be smaller compared to the effect of major stressful events, they occur much more frequently and can, therefore, have an important effect on general health. This project, therefore, focused at minor daily life stress and had two major objectives:

1) To study the affective and neuroendocrine sensitivity to daily life stress, conceptualized as minor activity-related stress (chapter 7).
2) To examine the role of genetic and environmental factors in individual differences in:
    i) Sensitivity associated with minor activity-related stress (chapter 8)
    ii) Distress associated with subclinical psychotic experiences, which also happen in the flow of daily life (chapter 5).

Minor activity-related stressful experiences in daily life were mapped using the *Experience Sample Method* (ESM). ESM is a structured diary technique used to assess subjects in their daily life environment, reducing biases in recall (Csikszentmihalyi and Larson, 1987; Delespaul, 1995; deVries, 1992). A trained research assistant visited each participant at home to explain the study procedures in detail. Subjects were instructed to wear a digital wristwatch programmed to emit a signal ("beep") at an unpredictable moment in each of ten 90-minute time blocks between 7:30 and 22:30, on five consecutive days. After each beep, subjects completed a short self-report form in a pocket-sized booklet, with open questions and rating scales concerning current thoughts, mood, activities and physical and social context (see appendix A). At the same time, participants took a saliva sample for cortisol determination. Salivary cortisol is a reliable indicator of the free cortisol in the plasma, which is considered to be the biologically active stress hormone. Cortisol rises when experiencing stress and stays measurable for a long time in saliva (Fuchs et al., 1997; Kirschbaum and Hellhammer, 1989; Kirschbaum and Hellhammer, 1994). ESM has already been successfully applied in different

Back to outline

psychiatric samples such as patients with schizophrenia (Delespaul, 1995; Myin-Germeys et al., 2001; Myin-Germeys et al., 2003), depression (Barge-Schaapveld et al., 1999; Peeters et al., 2004), bulimia (Larson and Asmussen, 1992), panic disorders (Dijkman-Caes and deVries, 1991) and general population samples (van Eck et al., 1996; van Eck et al., 1998). As mentioned above, the Experience Sampling method is a unique and ideal way to gather ecologically valid data, allowing investigating variability in relation to context. A possible drawback of this method is, however, the lack of control over participants' compliance with the method, as the self-reports are completed and the saliva samples are taken without supervision of the researcher. Chapter 6 of this thesis, therefore, investigated compliance with the intensive, random time sampling protocol for salivary cortisol, as used in this project, and examined indirectly the validity of momentary self-report data about daily experiences obtained with the same sampling methods. In chapter 7, the self-report data and the saliva samples, both collected according the ESM protocol, were used to study the pathways between minor, ongoing, activity related stress and changes in mood (positive affect, negative affect and agitation) and changes in the cortisol level.

Chapter 5, on the other hand, examined distress associated with subclinical psychotic experiences. Evidence is growing that psychotic experiences are much more common than originally assumed. Although only about 1% of the general population is identified as 'cases of psychosis', studies carried out in non-clinical populations have demonstrated that psychotic experiences (such as hearing voices) are quite common in these general population samples (Verdoux and van Os, 2002). For example, the Dutch Nemesis study revealed that about 17.5% of the general population endorsed at least 1 of the 17 Composite International Diagnostic Interview positive psychotic items (van Os et al., 2000). Studies using self-report questionnaires to explore psychotic experiences in non-clinical populations showed similar prevalence rates (Verdoux and van Os, 2002). In our project, subclinical psychotic experiences were assessed with the Community Assessment of Psychic Experiences (Stefanis et al., 2002). The CAPE is a self-report questionnaire, developed in order to rate self-reports of attenuated subclinical psychotic experiences in the affective and non-affective domains (see also http://www.cape42.homestead.com/). Furthermore, it has been shown that these experiences differ quantitatively, but not qualitatively, from psychotic experiences observed in patients (Johns and van Os, 2001). All previous work has focused on the occurrence and frequency of these subclinical psychotic experiences in the general population. However, it has been suggested that it is not only the frequency or intensity of subclinical psychotic experiences, but also the associated distress that is important in the development of clinical

Back to outline

need and patient status (Freeman et al., 2001; Freeman et al., 2002; Garety et al., 2001; Hafner, 2002; Hanssen et al., 2003; Krabbendam et al., in press; Peters et al., 1999). Chapter 5, therefore, focused on the distress associated with psychotic symptoms and investigated genetic and environmental influences on variation in associated distress.

As the second objective of this project was to examine individual differences in sensitivity to minor stress happing in the flow of daily life (conceptualized as i) minor activity-related stress and ii) subclinical psychosis-related distress), a twin sample was used. As showed in chapter 1, a twin study is a reliable method to disentangle genetic and environmental influences on the phenotype that is observed (i.e. stress-sensitivity). Our sample consisted of 289 adult female twin pairs (177 MZ and 112 DZ), and 47 of their sisters. Two hundred and twenty-nine pairs came from the East Flanders Prospective Twin Survey. Sixty pairs were recruited using registers from Flemish municipalities. Only female subjects were included in this study because self-reports of psychological symptomatology are found to be higher in females than in males, especially for self-reported depressive symptoms (Emslie et al., 1990; Fugita and Crittenden, 1990). In addition, studies also reported sex differences in stress-sensitivity. Men tend to report lower, less extreme levels of negative and positive affect, than women (Cameron, 1975; Diener et al., 1985) and were also found to expend greater effort in limiting emotional distress, resulting in lower emotional reactivity (Cysewski and Weiner, 1975). As the goal of this project was to examine individual variation of stress-sensitivity, and to link daily life stress-sensitivity to mental health, a female population was judged to be the most appropriate to achieve these objectives. Chapter 8 concentrated on the affective response to daily life stress (as measured with ESM) and examined to what degree individual differences in affective reactivity to daily life stress is explained by genetic and/or environmental factors.

Back to outline

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Back to outline

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Back to outline

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Back to outline

Chapter 3:

Heritability estimates of intelligence in twins: effect of chorion type

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Back to outline

ABSTRACT

This study investigates the basic assumption of homogeneity of monozygotic (MZ) twins: are there differences according to the timing of the zygotic splitting, early in dichorionic (DC) and later in monochorionic (MC) pairs? We assessed the IQ of 451 same-sexed twin pairs of known zygosity and chorion type with the Wechsler Intelligence Scale for Children-Revised (WISC-R). The variances of within-pair differences were compared for monochorionic (MC), dichorionic monozygotic (DC-MZ) and dizygotic same-sexed (DZ) twins and structural equation modeling was applied. High heritability estimates were found for almost all subscales and IQ-scores. A significant effect of chorion type was found: the MC twins resembled each other more than the DC-MZ twins on the subscales Arithmetic and Vocabulary. The effect accounts for respectively 14% and 10% of the total variance.

key words: **twins, chorion type, heritability, intelligence, prenatal environment**

Back to outline

INTRODUCTION

Monozygotic twins (MZ) arise from the division of a single fertilized ovum and are therefore genetically identical. According to differences in the antenatal development, three types of MZ twins can be distinguished. Approximately 32% of the MZ twins are dichorionic, where each fetus has its own chorion and amnion. Because the choriogenesis takes place around the fourth day after conception, dichorionic MZ twins (DC-MZ) probably have originated by a cleavage before the fourth day. Nearly 66% of the MZ twins are monochorionic-diamnionic (MC-DA). These twins share a common chorion, but have their own amnion. The separation must have taken place after the choriogenesis, but before the amniogenesis, which occurs after the seventh day of gestation. So separation probably occurred between the fourth and the seventh day of the gestation. Finally 2 to 3% of the MZ twins are monochorionic-monoamnionic (MC-MA), the two fetuses sharing one chorion and one amnion. In this case, the separation must have taken place after the amniogenesis, so after the eighth day (Leroy, 1991). The assumption that the division of the zygote occurs stepwise later in respectively DC-MZ, MC-DA and MC-MA pairs, has been recently demonstrated to be highly probable by studying X-inactivation in MZ female pairs : X-inactivation is totally symmetrical in MC-MA pairs, almost symmetrical in MC-DA pairs and asymmetrical in DC-MZ pairs (Monteiro *et al*., 1998; Puck, 1998; Chitnis *et al*., 1999). All dizygotic twins (DZ) - who result from the fertilization of two different eggs by two different spermatozoa - are dichorionic.

Monochorionic pairs almost always share the same placenta, in which, as a rule, large artery-to-artery and/or vein-to-vein vascular anastomoses allow the exchange of blood between both members of the pair (Benirschke and Kaufmann, 1995). Only exceptionally originates what is called the twin-to-twin-transfusion syndrome in which one-way arterio-venous anastomoses direct the blood to flow from one twin to the other and in which the balance is not re-established because of the absence or the small size of the large anastomoses. In its most severe form, this can cause the death of one or both twins (Schatz, 1882-1910; Strong and Corney, 1967). Another characteristic of the monochorionic placenta is the occasional unequal sharing of placental tissue, leading to growth retardation of one member of the pair (Machin *et al.,* 1995).

Although some have assumed that chorion type may influence postnatal phenotypes (Phillips 1993), only few have investigated its effect. Compared with DC-MZ twins and DZ twins, MC twins have a higher perinatal mortality rate (Derom *et al.,* 1991). The mean birth weight of twins is highest in DZ pairs, somewhat lower in DC-MZ pairs, but significantly lower in MC

Back to outline

pairs (Derom *et al*., 1995; Loos *et al*., 1998). Mean within-pair birth weight differences are lowest in DC-MZ twins. In MC and DZ pairs, the differences are larger, but of approximately the same magnitude in both groups (Vlietinck *et al.,* 1989; Derom, 1994; Spitz *et al.,* 1996). The sex proportion, that is the proportion of males to all newborns, is slightly lower in MZ than in DZ twins (Derom *et al.,* 1988; Loos *et al.,* 1998). Among MZ twins, the sex proportion of the DC and the MC-DA pairs is similar, whereas the sex proportion of the few MC-MA pairs is significantly lower (0.23). Finally, congenital anomalies, especially cardiac malformations, are more frequent in MC as compared to DC-MZ pairs (Cameron *et al*., 1983).

Furthermore, influences of chorion type have been studied on measures of intelligence and personality. In several studies, an effect of chorion type on cognitive measures was found. In 7-year-old caucasian twins, the mean square of within-pair differences of IQ is greater in DC-MZ than in MC twins, indicating that DC-MZ twins are more discordant than MC twins (Melnick *et al.,* 1978). MC twins are more similar than DC-MZ twins on the Block Design subtest of the WAIS (Rose *et al.,* 1981) and of the WISC-R (Spitz *et al.,* 1996). In a three-year follow-up study of the last study (Gutknecht *et al.,* 1999), the effect of chorion type on the Block Design subtest could not be replicated. However, no relationship was found between chorion type and scores on the Bayley scales in 18-month-old twins (Welch *et al.,* 1978), IQ-scores on the Stanford-Binet test in 4-year old twins (Brown, 1977), scores on the McCarthy Scales of Cognitive Ability in 4- to 6-year-old twins (Sokol *et al.,* 1995) and scores on the Digit Symbol Substitution test in adults (Reed *et al*., 1991). In none of the studies was the full WISC-R battery administered to the subjects. In a study on personality measures, greater within-pair differences in DC-MZ than in MC twins were found (Sokol *et al*., 1995).

The aim of this study was to determine the influence of chorion type on heritability estimates of IQ-measures in an extensive sample of same-sexed twins of known zygosity and chorionicity from a population-based register, applying structural modeling.

Back to outline

SUBJECTS & METHODS

Subjects

Since 1964, the "East Flanders Prospective Twin Survey" (EFPTS) collects information on the mother, the placenta and the child of 98% of the multiples born in the province of East Flanders, Belgium (Loos *et al*., 1998). At the end of 1998, the register counted 5371 twin pairs, 196 sets of triplets and 27 sets of higher order multiples**.** Zygosity of all twins was determined through sequential analysis based on sex, foetal membranes, umbilical cord blood groups (ABO, Rh, CcDEe, MNSs, Duffy, Kell), placental alkaline phosphatase and, since 1982, DNA fingerprints (Loos et al, 1998). Unlike-sex twins and same-sex twins with at least one different genetic marker were classified as DZ; monochorionic twins were classified as MZ. For all same-sex dichorionic twins with the same genetic markers a probability of monozygosity was calculated using a lod-score method (Vlietinck, 1986; Meulepas et al, 1988).

The participants came from two studies conducted by the EFPTS-team. In the first study 54 pairs of MZ twins between the ages of 9 and 11 were selected from a consecutive series. Both children had to be alive and not suffering from a severe mental retardation. The sample was balanced for sex and chorion type: 25 male and 29 female, 28 monochorionic and 26 dichorionic pairs. Sex, chorion type and age were independent from each other. The second study was a project on mental retardation in twins. All twins from the EFPTS register born between September 1982 and December 1991 from Belgian ancestry were invited to participate. The study sample was representative for gender, birth weight and gestational age. As in most of the twin studies, the MZ twins were slightly overrepresented due to self-selection biases. In total 618 twin pairs between the ages of 8 and 14 have been tested: 161 MC, 80 DC-MZ, 188 DZ same-sexed and 189 DZ opposite-sexed twins. Eleven pairs of twins in the second study (4 MC-MZ, 7 DC-MZ) were also tested in the first study four years earlier. Their score on the second test was used. For the analyses on chorionicity, only complete same-sexed pairs were used, as the intra-pair differences were examined (28 incomplete pairs (10 MC-MZ, 4 DC-MZ, 7 DZ liked-sexed, 7 DZ unliked-sexed) and 182 DZ unlike-sexed pairs were excluded). The final sample consisted of 175 MC, 95 DC-MZ and 181 DZ same-sexed pairs, of which 223 were male and 228 female pairs. Of the 95 DC-MZ twins, 88 reached a probability of monozygosity of at least 0.99 and 7 reached a probability of monozygosity of at least 0.95**.**

Back to outline

Measures

All twins completed the Wechsler Intelligence Scale for Children-Revised (WISC-R). It consists of 6 verbal and 6 performance subtests (Wechsler, 1986). The verbal subtests are Information (INF), Similarities (SIM), Arithmetic (ARI), Vocabulary (VOC), Comprehension (COM) and Digit Span (DS). The performance subtests are Picture Completion (PC), Picture Arrangement (PA), Block Design (BD), Object Assembly (OA), Coding (COD) and Mazes (MAZ). The scores on the subtests are standardized for age and added up to Verbal (VIQ), Performance (PIQ) and Total Intelligence Quotients (TIQ).

Analyses

The means and variances of MC, DC-MZ and DZ twins were compared by unpaired t-tests and F-tests, respectively, for the firstborn and the second born children separately. The descriptive analyses were done with SAS (SAS Institute, 1997). The within-pair difference in scores was calculated. The variances of the within-pair differences in MC, DC-MZ and DZ twins were compared by an F-test**,** corrected for multiple testing. Next, structural equation modeling was applied to the data. Using the program Mx (Neale, 1999), models composed of an additive genetic effect (A), common environment (C), unique environment (E) and chorion type (Ch) were fit to the observed covariance matrices. The correlation coefficient between the A components of the two children is 1 for MZ and 0.5 for DZ twins, for C the correlation is 1. As we assumed a higher similarity between MC than between DC twins, the correlation between the Ch components was set to 1 for MC and 0 for DC twins (Figure 1). The choice between alternative models was made by their maximum likelihood estimation and their parsimony, measured by the Akaike Information Criterion (AIC) (Akaike, 1987), which combines the chi² with the degrees of freedom (df), (Williams and Holahan, 1994): $AIC=\chi^2-2df$. Under the best fitting model, the parameters and their 95% confidence intervals (C.I.) were estimated. In case a model was chosen on the basis of having the lowest AIC, but the C.I. of one of the estimated variance components contained 0, the model without that component was preferred. A likelihood ratio chi-square test of the chosen model against the full model was performed for each measure.

A separate heritability estimation was also computed based on the comparison of DZ twins with MC and DC MZ twins.

Back to outline

RESULTS

The means and standard deviations of the subscales and the IQ-scores are listed in Table 1. There were no differences in means between MC, DC-MZ and DZ twins. The firstborn MC have a higher variance for Similarities (p=0.02) and Comprehension (p=0.02) when compared to DC-MZ twins. Second-born MC twins have a lower variance for Vocabulary than DZ twins (p=0.04). Comparing DC-MZ and DZ twins, firstborn DZ twins have a higher variance for Comprehension (p=0.02).

The intra-class correlations and the variances of the within-pair differences for MC, DC-MZ and DZ twins were calculated and the within-pair variances compared by F-tests (Table 2). The within-pair variances of MC twins were significantly smaller than the within-pair variances of DC-MZ twins for Arithmetic, Vocabulary and Comprehension. However, after correction for multiple testing, only Vocabulary remains significant. For the other measures, the within-pair variances of MC and DC-MZ were not different. The within-pair variance of DC-MZ twins was smaller than that of DZ twins for all tests, except for Picture Completion, Object Assembly and Mazes.

Model fitting.

All tested models were nested under the full 4-parameter model. Table 3 shows for each measure the best fitting model and the likelihood ratio chi-square of that model against the full model. A model specifying an additive genetic component and a unique environment, an AE-model, was preferred for Information, Similarities, Comprehension, Digit Span, Picture Completion, Picture Arrangement, Block Design, Coding, Mazes, Performance IQ, Verbal IQ and Total IQ. An AE-model with a component of chorion type was the best fitting model for Arithmetic and Vocabulary. Finally an ACE-model, specifying a genetic component and both common and unique environment, was best fitting for Object Assembly.

The estimates and the 95% C.I. of the model parameters are described in Table 3. The heritability estimates range from 29% (C.I. 19-39%) for Picture Completion to 83% (C.I. 79-86%) for Total IQ. The proportion of variance explained by chorion type is 14% (C.I. 1-29%) for Arithmetic and 10% (C.I. 2-19%) for Vocabulary.

Back to outline

DISCUSSION

The mean scores were not different between MC, DC-MZ and DZ twins. For the variances some minor differences were found. However, the effects are small and could be false positive results, due to the large number of tests performed.

A general finding is the high heritability found for almost all measures. The heritability of Total IQ was 83% in our sample, with C.I. ranging from 79 to 86%. Our results regarding the heritabilities of intelligence are in agreement with numerous previous twin studies (Fulker and Cardon, 1983; Boomsma, 1993). The age-old "nature vs. nurture" debate about cognitive development is not going to settle down. The reader is referred to the excellent report on the diversity of views written by Baker at the Dahlem workshop on twins as a tool of behavioural genetics (Baker et al., 1993).

The results from the comparison of the variances of the within-pair differences roughly correspond to the output of the model fitting. For subtests where the within-pair variances in the MC pairs were equal to the variances in the DC-MZ pairs, but smaller than that in DZ pairs, an AE-model was found. This corresponds to the classic twin design, where one assumes that the MZ pairs, as a homogeneous group, resemble each other more than the DZ twins.

A different finding emerged for Arithmetic and Vocabulary. Here the MC pairs were more similar than the DC-MZ pairs, which in turn were more alike than the DZ twins were. This pattern of within-pair variances suggests the combined action of genetics and chorion type. The best fitting model for Arithmetic and Vocabulary was indeed an AE-chorion model. The proportion of variance explained by chorion type was significant and explained respectively 14% and 10%.

For Comprehension the DC-MZ twins resembled each other better than MC twins did, which in turn were more alike than DZ twins. The best fitting model for this subtest however was an AE-model. We tested a model in which the effect of chorion type was reversed, so that the DC-MZ would be more alike than the MC, but no effect could be detected.

A last pattern of within-pair variances, in which no differences between MC, DC-MZ and DZ were found, was observed in Picture Completion, Object Assembly and Mazes. For Picture Completion and Mazes an AE-model fits the data best, while for Object Assembly the best fit came from the ACE-model. The estimate of the genetic component was low for these tests: 29%, 37% and 24% respectively.

Back to outline

To get a clearer picture of the influence of chorion type on estimates of heritability, we performed a separate modeling of MC vs. DZ twins and of DC-MZ vs. DZ twins and looked at the heritability estimates resulting from these models. In the group of MZ twins who are MC, the heritability estimates of the subtests showing a chorion effect were slightly, but not significantly higher than in the MZ group consisting of only DC twins. For Arithmetic the estimates were 67% (C.I. 59-74%) when comparing MC and DZ twins and 54% (C.I. 40-65%) when comparing DC and DZ twins. For Vocabulary the estimates were respectively 82% (C.I. 77-86%) and 73% (C.I. 64-80%). This suggests that when analyzing a trait that is under influence of chorion type, the composition of the MZ group could be important for the accuracy of the heritability estimate.

The 12 WISC-R subtests can be reduced to an orthogonal 3-factor structure (Kaufman 1975). The two subtests that indicated a chorion effect, however, belong to different factors. Vocabulary forms part of "Verbal Comprehension" and Arithmetic falls under "Freedom from Distractibility". The tests of Vocabulary and Arithmetic also have something in common: they are both highly correlated with general intelligence. In Vocabulary the knowledge of words and the level of abstract thinking are tested. In Arithmetic the level of mastering the basic arithmetic operations is measured. These skills are all closely related to the home and school environment. In addition, more than the other tests, Arithmetic is sensitive to swings in mood or attention.

It is further worthwhile to notice that, although the specific cognitive abilities as measured by the different subtests show substantial genetic influence, their genetic contribution is less than for total IQ (Plomin and Defries, 1998). The different subtests are all correlated and multivariate genetic analyses have shown that the genetic correlations among these specific cognitive abilities can be very high (Petrill et al., 1997). Finding chorion effects on the more specific cognitive abilities such as Arithmetic and Vocabulary is important to understand the underlying mechanisms of a more general cognitive ability as is measured by total IQ.

How can the chorion effect that is found be linked to the biology of twinning? The major anatomical difference between MC and DC twins is that the MC almost always share the same placenta and have a common blood circulation. In the theory of "fetal programming", it is stated that during fetal growth and development, the various tissues of the body grow during different critical periods of rapid cell division. Furthermore, changes in the nutrient and hormonal milieu of the conceptus at these times may alter expression of the fetal genome, leading to permanent effects on a range of physiological processes (Godfrey, 1998). So, as the

Back to outline

MC twins share their blood circulation, a similarity in fetal programming could explain the greater concordance in some characteristics compared to DC-MZ twins.

The studies that found no difference in within-pair variance between MC and DC twins were conducted on very small samples of young children or adults. This could suggest that age could be a mediating factor. In our sample the children are between 8 and 14 years old. The ages of 8 to 14 are crucial years of puberty, in which drastic hormonal changes occur. It could be that these hormonal changes differentiate children, even MZ twins, except in the case of monochorionicity, where the children prenatally share a common vascular system. In addition, in some of the other studies the diagnosis of chorionicity was established based on dermatoglyphs, which is demonstrated to be an unreliable method (Reed et al., 1997).

Finally, the effect of chorion type that we found may represent a “maternal effect” of the kind suggested by Devlin et al (1997) in their meta-analysis of 212 studies on the heritability of IQ. In this analysis, 20% of the variance in IQ could be explained by such “maternal effects”. We think chorion type could be such an effect.

The finding that for some phenotypes MC twins are more similar than DC-MZ twins, is of major importance for the basic methodology used in twin studies. In general, MZ twins are treated as a homogeneous group. In fact three types of MZ twins exist, depending on the time of splitting which is reflected by the placental structure.

The comparative estimation of heritability of IQ measures according to the chorion type has to do with the basic assumption of the classic twin study. As already pointed out half a century ago by Price (1950), "prenatal and natal difference producing factors in monozygotic twins should be considered as biases in the estimation of the significance of heredity in the medical and behavioural sciences". The major prenatal factor is the peculiar environment of two thirds of the monozygotic twins, characterized by their mutual blood circulation. A previous study (Melnick et al., 1975) of the genetic variance of IQ, a study in which the chorion type was taken into account , was based on the general model for the estimate of quantitative twin data as described by Christian and colleagues (Christian et al., 1974). From the analysis of their sample of white twins, Melnick et al. concluded that the zygosity and/or chorion type had little effect on the overall mean IQ. But the observed within-pair measure of IQ indicated that members of the 9 MZ-DC pairs were significantly more discordant than the members of the 23 MZ-MC pairs. Our sample was larger and we used a structural model to analyse the data, whereas the validity of the Christian et al. model has been questioned (Nance, 1974). We were able to side-step the chorion prenatal bias by contrasting a sizeable sample of MZ and DZ twins whose antenatal development has been comparable as far as the

Back to outline

placental structure is concerned. One of the major basic assumptions of the classic twin study is herewith fulfilled, i.e. no intrauterine environment differences between the two types of twins.

It is interesting to note that the slight differences in heritability estimates tend to be higher when the monochorionic group is compared to the DZ group. Possibly, the monochorionics could be more alike because antenatally they live in closer contact with each other and exchange their blood through placental vascular anastomoses, which may be important for their nutrition, the transport of toxins, hormones and other agents which can influence brain development.

It should further be noted that what we have found regarding the heritability of IQ measurements may not be applicable to other domains. Looking at within-pair birth weight differences according to chorion type we showed that there was a significant effect of chorion type going in the opposite direction as what was found in this study : the effect of chorion type explained 12% of the total variance but here DC-MZ twins resembled each other more than MC twins (Vlietinck *et al*., 1989). So, according to the fetal-origin hypothesis (Godfrey, 1998) the prediction would be that heritability estimates of phenotypes, which are influenced by birth weight and suboptimal intrauterine nutrition, will be higher when the DC-MZ group is compared to the DZ group.

Another important bias in the classic twin study is the unequal lyonisation in female MZ twins, which has been shown to occur only in DC-MZ twin girls (Monteiro *et al*., 1998). Again, chorion type seems to be important according to within-pair differences in X-inactivation and therefore in estimating the genetic component of X-linked traits in twin girls.

Finally, although an effect of chorion type was found on two subscales, it must be said that no effect was found on total IQ. Furthermore, the reported effects are small with confidence intervals close to zero and are not (yet) replicated. So, further studies along the same line need to be done because of inconsistency across those studies that focused on chorion type.

Back to outline

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Back to outline

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Back to outline

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Back to outline

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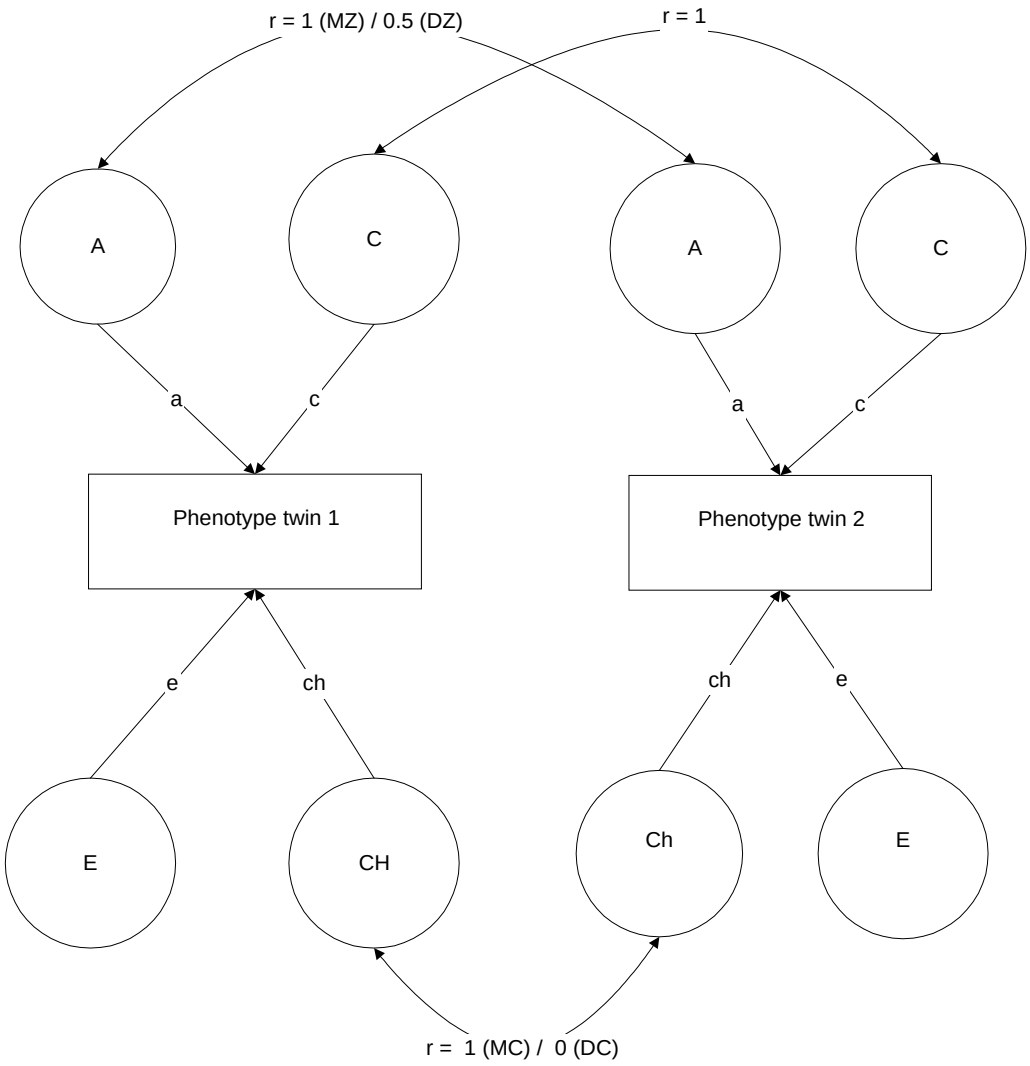
Back to outline

Figure 1. Structural equation model used in the program MX



Back to outline

Table 1. Means and standard deviations of WISC-R subtests and tests for MC, DC-MZ and DZ twins

	MC				**DC-MZ**				**DZ**			
WISC-R (sub)tests	twin 1		twin 2		twin1		twin 2		twin 1		twin 2	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
INF	8.9	2.6	8.8	2.6	9.1	3.0	9.0	2.6	9.0	2.9	8.8	2.7
SIM	11.0	3.1	10.7	2.9	10.8	2.5	10.8	3.3	10.9	3.0	10.8	3.1
ARI	9.8	2.9	10.0	2.9	9.6	2.8	10.1	2.7	10.1	3.0	10.2	3.1
VOC	11.0	2.6	11.0	2.5	11.3	2.6	11.3	2.8	11.3	2.8	11.4	2.9
COM	11.3	3.0	11.7	2.9	11.3	2.4	11.1	2.8	11.3	3.0	11.1	3.0
DS	10.3	3.2	10.4	3.0	10.6	2.9	10.3	3.0	10.4	2.9	10.4	3.1
PC	10.1	2.9	10.0	3.2	10.0	2.7	10.4	3.3	10.2	2.8	10.1	2.9
PA	10.1	2.8	9.8	2.9	10.2	2.8	10.0	2.9	10.0	3.1	10.1	3.1
BD	9.9	3.1	10.0	2.8	10.6	3.0	10.5	3.3	10.2	3.2	10.3	3.1
OA	9.0	3.4	9.4	3.1	9.8	3.7	9.8	3.2	9.3	3.3	9.2	2.9
COD	10.2	3.2	10.2	3.0	10.4	3.1	10.4	3.0	10.5	3.3	10.3	3.0
MAZ	10.4	3.0	10.3	2.9	9.9	2.7	10.2	3.0	10.5	2.9	10.7	2.9
VIQ	102.7	14.8	102.9	13.9	102.9	13.3	103.0	14.4	103.4	15.1	103.1	15.1
PIQ	99.8	15.8	99.8	14.0	101.5	14.8	101.9	15.8	101.1	15.7	101.3	15.2
TIQ	101.6	15.2	101.6	13.5	102.4	14.3	102.7	15.2	102.5	15.1	102.5	15.1

Back to outline

Table 2. Correlations and within-pair variances of WISC-R subtests and tests for MC, DC-MZ and DZ twins and F-test of variances

WISC-R	correlations			variances			MC vs. DC-MZ			DC-MZ vs. DZ		
(sub)tests	MC	DC-MZ	DZ	MC	DC-MZ	DZ	F	p		F	p	
INF	0.69	0.69	0.49	3.83	4.99	8.35	1.30	0.07		1.68	0.003	*
SIM	0.50	0.50	0.22	9.27	9.11	14.93	1.02	0.47		1.64	0.004	*
ARI	0.66	0.49	0.34	5.66	7.95	13.08	1.40	0.03		1.65	0.004	*
VOC	0.77	0.70	0.46	2.64	4.34	9.08	1.64	0.003	*	2.09	<0.001	***
COM	0.51	0.55	0.33	8.38	6.15	12.05	1.36	0.05		1.96	<0.001	**
DS	0.48	0.56	0.38	9.26	7.34	11.73	1.26	0.1		1.60	0.006	*
PC	0.36	0.22	0.16	12.53	14.18	14	1.13	0.24		1.01	0.46	
PA	0.45	0.53	0.42	9.07	7.46	12.35	1.22	0.15		1.66	0.004	*
BD	0.67	0.74	0.34	6.15	4.67	13.41	1.32	0.07		2.87	<0.001	***
OA	0.56	0.54	0.39	9.98	11.07	12.1	1.11	0.28		1.09	0.32	
COD	0.67	0.65	0.39	6.28	6.48	12.78	1.03	0.42		1.97	<0.001	**
MAZ	0.40	0.32	0.13	10.14	11.23	14.8	1.11	0.28		1.32	0.07	
TVIQ	0.82	0.79	0.43	73.88	81.2	256.88	1.10	0.29		3.16	<0.001	***
TPIQ	0.73	0.73	0.43	124.04	126.71	272.18	1.02	0.45		2.15	<0.001	***
TIQ	0.83	0.82	0.44	73.18	78.89	254.7	1.08	0.33		3.23	<0.001	***

* p<0.05; ** p<0.01; ***p<0.003 (corrected for multiple testing)

Back to outline

Table 3. Estimates and 95% C.I. of genetic, environmental and chorion parameters under the full 4-parameter model and under the best fitting model

	Model	chi²	df	p	AIC	p(chi²test)	a	95% C.I.	c	95% C.I.	Ch	95% C.I.	e	95% C.I.
INF	ACE-ch	6.52	5	0.26	-3.48	0.11								
	AE	10.88	7	0.14	-3.12									
SIM	ACE-ch	11.18	5	0.05	1.19	0.96								
	AE	11.26	7	0.13	-2.74		0.48	0.39-0.57	-	-	-	-	0.51	0.43-0.61
ARI	ACE-ch	1.98	5	0.85	-8.02	0.79	0.49	0.1-0.64	0.04	0-0.32	0.15	0.01-0.-32	0.33	0.26-0.41
	AE-ch	2.05	6	0.92	-9.96		0.53	0.4-0.64	-	-	0.14	0.01-0.29	0.33	0.26-0.41
VOC	ACE-ch	5.06	5	0.41	-4.94	0.26	0.58	0.3-0.78	0.13	0-0.35	0.11	0.03-0.22	0.18	0.14-0.23
	AE-ch	6.31	6	0.39	-5.69		0.72	0.63-0.79	-	-	0.10	0.02-0.19	0.18	0.14-0.23
COM	ACE-ch	8.31	5	0.14	-1.69	0.80	0.46	0.17-0.62	0.09	0-0.33	0	0-0.1	0.46	0.38-0.54
	AE	8.76	7	0.27	-5.24		0.55	0.47-0.62	-	-	-	-	0.45	0.38-0.53
DS	ACE-ch	3.77	5	0.58	-6.24	0.36	0.34	0.05-0.6	0.19	0-0.43	0-	0-0.1	0.47	0.39-0.55
	AE	5.79	7	0.57	-8.21									
PC	ACE-ch	6.36	5	0.27	-3.64	0.6								
	AE	7.38	7	0.39	-6.62		0.29	0.19-0.39	-	-	-	-	0.71	0.61-0.81
PA	ACE-ch	3.6	5	0.61	-6.4	0.47	0.34	0.04-0.58	0.16	0-0.39	0	0-0.11	0.50	0.42-0.59
	AE	5.10	7	0.65	-8.90		0.52	0.43-0.59	-	-	-	-	0.48	0.4-0.57
BD	ACE-ch	9.23	5	0.1	-0.77	1	0.70	0.52-0.75	0	0-0.17	0	0-0.06	0.3	0.25-0.37
	AE	9.23	7	0.24	-4.77		0.7	0.63-0.5	-	-	-	-	0.3	0.25-0.37
OA	ACE-ch	11.49	5	0.04	1.49	0.76	0.21	0-0.54	0.29	0-0.51	0.02	0-0.19	0.48	0.39-0.57
	ACE	11.58	6	0.07	-0.43									
COD	ACE-ch	3.23	5	0.66	-6.77	0.91								
	AE	3.41	7	0.85	-10.59		0.67	0.61-0.73	-	-	-	-	0.32	0.27-0.39
MAZ	ACE-ch	2.62	5	0.76	-7.38	0.48	0.3	0-0.44	0	0-0.25	0.12	0-0.32	0.58	0.48-0.71
	AE	4.10	7	0.77	-9.91		0.37	0.27-0.46	-	-	-	-	0.63	0.54-0.73
TVIQ	ACE-ch	4.58	5	0.47	-5.42	0.84	0.78	0.55-0.85	0.03	0-0.24	0.02	0-0.09	0.17	0.14-0.22
	AE	4.92	7	0.67	-9.08		0.82	0.78-0.85	-	-	-	-	0.18	0.15-0.22
TPIQ	ACE-ch	6.35	5	0.27	-3.65	0.63	0.61	0.34-0.77	0.12	0-0.34	0	0-0.11	0.27	0.21-0.33
	AE	7.27	7	0.40	-6.73		0.73	0.68-0.78	-	-	-	-	0.27	0.22-0.32
TIQ	ACE-ch	8.6	5	0.13	-1.40	0.89	0.78	0.55-0.86	0.04	0-0.25	0.01	0-0.08	0.17	0.13-0.21
	AE	8.83	7	0.27	-5.17		0.83	0.79-0.86	-	-	-	-	0.17	0.14-0.21

Chapter 4:

Child psychopathology and lower cognitive ability:

A general population twin study of the causes of association

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Back to outline

ABSTRACT

Previous work has demonstrated associations between lower cognitive ability and childhood and adult non-psychotic psychopathology. As both cognitive ability (CA) and child psychopathology (CP) are influenced by genetic factors, one explanation for the association is that they are the pleiotropic manifestations of the same underlying genetic factors. The present paper examines three possible causes of the association: additive genetic factors, common environmental factors and individual-specific environmental factors.
376 twin pairs from the East Flanders Prospective Twin Survey were examined with the Child Behaviour Checklist and the Wechsler Intelligence Scale for Children-Revised. The cross-twin within-variable, within-twin cross-variable and cross-twin cross-variable correlations were calculated. Using structural equation modeling, bivariate models were fitted. The best fitting model was chosen, based on likelihood and parsimony.
The observed phenotypic correlation between CP and CA was –0.19 (95% CI:-0.09,-0.27), with genetic factors accounting for about 84% of the observed correlation. Bivariate model fitting quantified the genetic correlation between CP and CA at –0.27 (95% CI:-0.12,-0.42) and the individual-specific environmental correlation at –0.17 (95% CI: -0.03,-0.31).
In children, three different genetic factors may exist: one that solely affects the liability to CP, one that has only an effect on CA and one that influences both CP and CA. While individual-specific environmental factors can influence the liability to both traits, our results suggest that most of the environmental factors that increase the risk of CP do not influence CA and vice versa.

Keywords **:** CBCL, IQ, twins, bivariate genetic analysis, children

Back to outline

INTRODUCTION

Several studies have examined the relationship between measures of cognitive ability and psychopathology. The association between these two traits has been found to be stable over time (1) (2) (3) and has been reported in clinical samples (4) (5) as well as in population-based samples (6) (7). Two general population cohort studies independently found that lower childhood cognitive ability was associated with non-psychotic psychopathology in adulthood (8) (9). However, the earliest origin of this association appears to be in childhood (8). In addition, there is work linking lower intelligence and specific childhood psychopathologies such as hyperactivity (10) and delinquency (11). Childhood psychopathology and lower cognitive ability may be associated because one is a risk factor for the other, and/or because both are determined by a third cause. In this study, we wished to examine to what degree childhood psychopathology and lower cognitive ability as expressed by IQ scores are determined by the same causal factors.

Twin studies have shown a significant influence of genetic, shared environmental and non-shared environmental factors on child psychopathology as measured by the Child Behaviour Checklist (CBCL) (12) (13) and as measured by other instruments including direct interviews (14) (15). Other twin studies have demonstrated that genetic factors also influence intelligence expressed as IQ scores (16) (17) (18). Common environmental influences on IQ play a role in early childhood and tend to diminish with age. After childhood, IQ appears to be influenced only by genetic and non-shared environmental factors (16) (19) (20). As child psychopathology (hereafter: CP) and lower cognitive ability (hereafter: CA) co-occur and both traits are influenced by genetic and environmental factors, it is attractive to speculate that part of the observed association between CP and CA is caused by the same genes and/or environments. In order to test this hypothesis, a request was made to use the data of a population-based twin sample in East Flanders, Belgium.

METHODS

Subjects

The East Flanders Prospective Twin Survey (EFPTS) has recorded multiple births in the province of East Flanders (Belgium) since 1964. Basic perinatal data such as birth weight, gestational age, mode of delivery, and placentation are collected. The zygosity of the twins is determined by sex, placentation, blood groups and, since 1982, by examination of five highly polymorphic DNA markers. Unlike-sex twins and same-sex twins with at least one

Back to outline

different genetic marker were classified as dizygotic; monochorionic twins were classified as monozygotic. For all same-sex dichorionic twins with the same genetic markers a probability of monozygosity was calculated using a lod-score method (21). After DNA fingerprinting, a probability of monozygosity of 0.999 was reached. To be included in the analysis, dichorionic MZ pairs had to reach a probability of monozygosity of at least 95%. So far, the register has collected information on more than 5600 pairs of twins (22).

The data used in this paper were collected in two different sampling frames. In the first, 1436 twins aged 6–17 years were selected from the EFPTS. Their parents were asked to fill in the Child Behaviour Checklist (CBCL). Seven hundred and sixty parents (53% of the total available parents) participated. Eight pairs were excluded because of major congenital malformations (6 pairs), missing zygosity (1 pair) or implausible birth weight data (1 pair). Of the 752 pairs, 425 (57%) were dizygotic (DZ) while 327 (43%) were monozygotic (MZ).

The second sampling frame was related to a project on mental retardation. Twins from the EFPTS born between September 1982 and December 1991 were invited to participate. In total, 663 twin pairs aged 8-14 years were tested: 286 (43%) MZ and 377 (57%) DZ pairs. Of the 663 pairs, 376 had also participated in the first study in which the CBCL had been used: 168 (45%) MZ and 208 (55%) DZ. In order to examine the representativeness of these 376 twin pairs in terms of sex, birth weight, zygosity, mode of fertilization, gestational age, parental ages, we compared this group with all twins registered in the EFPTS born alive between 1980 and 1989 (Table 1). This revealed that the 376 twin pairs were representative in terms of gender, mode of fertilization, gestation and parental age. However, the MZ pairs were slightly overrepresented (due to self-selection bias in the mental retardation project (18)) and the second difference was that birth weight was slightly higher in the group of 376 pairs. In order to examine the representativeness of the 376 twin pairs in terms of CBCL and IQ, we compared this group with the non-overlapping twin pairs (CBCL: 749 children, IQ: 546 children) from the two sampling frames of the original CBCL and mental retardation studies (Table 1). This revealed that IQ scores were slightly higher in the study sample than in the excluded sample. However, there were neither large nor significant differences in CBCL score.

Back to outline

Measures

The CBCL was developed by Achenbach (1991) to examine the extent to which children have behavioural and emotional problems as perceived by their parents. Although the CBCL allows for the calculation of separate scores corresponding to several behavioural dimensions based on exploratory factor analysis, confirmatory factor analytic studies have shown inadequate empirical support for these syndromes and their differentiation (23, 24). Instead, a general problem behaviour factor appears to underlie CBCL data across different age groups (23, 24). We therefore examined the total amount of psychopathology, as measured by the total problem score, subjected to a square-root transformation to achieve normality.

In the second study, all twins completed the full Wechsler Intelligence Scale for Children-Revised (WISC-R)(25), consisting of 6 verbal and 6 performance subtests. This test was administered separately by two trained research workers. The scores on the subtests were standardized for age and added up to Verbal, Performance and Total Intelligence Quotients.

Analysis

Three types of analyses were carried out. First, three kinds of correlations were calculated, using STATA (26): cross-twin within-variable (ie, CP in twin 1 correlated with CP in twin 2 and same for CA: r_{cp}, r_{ca}), within-twin cross-variable (ie, CA in twin 1 correlated with CP in twin 1 and same for twin 2: r_p), and cross-twin cross-variable (ie, CP in twin 1 correlated with CA in twin 2 and vice versa: r_{xt}) correlations. The causes of association can usually be intuited from the pattern of these correlations in MZ and DZ twins. The comparison between r_{cp} and r_{ca} in MZ and DZ twins gives an impression about the role of additive genetic factors (A), common environmental factors (C) and individual-specific environmental factors (E) in CP and CA separately. In addition, if r_{xt} in MZ twins is significantly higher than r_{xt} in DZ twins, a genetic factor can be hypothesized to play a role in the association between the two traits. Furthermore, if all of the association is due to genes, then r_p in MZ twins should be the same as r_{xt} in MZ twins. If, on the other hand, r_p in MZ twins is higher than r_{xt} in MZ twins, individual environmental factors are also likely to contribute to the association.

Second, univariate structural equation models were fit to the whole CBCL dataset (first twin study; n = 752 pairs) and to the whole IQ dataset (second study, n = 663 pairs). Univariate structural equation modeling decomposes the variance within a phenotype into three

Back to outline

possible sources: (1) genetic factors, (2) common environmental factors and (3) unique environmental factors. The results of the univariate modeling procedures were used to guide the bivariate models described below.

Third, based on the assumptions resulting from the pattern of the first two analyses, structural equation modeling, using Mx (27), was used to fit bivariate models to the whole dataset. The total IQ score was divided by ten, in order to take into account scale differences between CBCL and WISC-R. The goal of a bivariate twin analysis (Figure 1) is to decompose the covariance between two associated characteristics (CP and CA) into three possible sources: (1) genetic factors, (2) common environmental factors (those environmental experiences that are shared by both members of a twin pair), (3) individual-specific environmental factors (those environmental experiences not shared by both members). Several models were fitted to the data. The models were compared using the difference in fits and the difference in degrees of freedom as criterion (28). The best fitting model was chosen, based on likelihood and parsimony of the model. The 95% confidence intervals were calculated (29). The bivariate heritability (that part of the phenotypic correlation that is due to shared genes: $\sqrt{a^2_{cbcl}} \times r_a \times \sqrt{a^2_{wisc-r}}$), the bivariate c^2 (that part of the phenotypic correlation that is due to shared environmental factors: $\sqrt{c^2_{cbcl}} \times r_c \times \sqrt{c^2_{wisc-r}}$) and the bivariate e^2 (that part of the phenotypic correlation that is due to non-shared environmental factors: $\sqrt{e^2_{cbcl}} \times r_e \times \sqrt{e^2_{wisc-r}}$) were calculated.

RESULTS

Sample

The main correlational and bivariate analyses were done on a sample of 376 twin pairs: 168 MZ and 208 DZ pairs. The group consisted of 129 same-sex male pairs, 150 same-sex female pairs and 97 opposite sex pairs. The mean age at which the CBCL was completed by the parents was 8.2 years (SD=1.84) and the child was tested with the WISC-R a mean of 2.9 years later (SD=0.90). Mean age at that time was 11.1 years (SD=1.50). The IQ data of 56 children (7.4 % of the total sample) was obtained around 2.6 years before the CBCL data. The mean total IQ within the sample was 104.22 (SD=14.29), the mean verbal IQ was

Back to outline

104.72 (SD=13.87) and the mean performance IQ was 102.73. (SD=14.54). The mean (transformed) score on the CBCL was 3.99 (SD=1.64).

Correlations

Univariate

A significant association between CP, assessed by the CBCL, and CA, assessed by the WISC-R, was found for the firstborn twin ($r = -0.16$; $p < 0.05$) as well as for the second born child ($r = -0.19$; $p < 0.001$). The correlations (Table 2) in MZ pairs for CP ($r = 0.79$) and CA ($r = 0.80$) were substantially higher than those observed among DZ pairs ($r = 0.57$ and $r = 0.53$, respectively), suggesting that genetic factors play a causative role in CP and CA separately.

The fact, however, that r_{ca} and r_{cp} among DZ twins were higher than half of those among MZ twins suggests that they are also under the influence of environmental factors shared by the pairs.

Bivariate

First, the difference between the MZ r_p and DZ r_p was examined. Using Mx (27), it was formally tested if within each group (MZ and DZ) r_p could be equated for twin 1 and twin 2. The difference in fit was not significant ($\Delta\chi^2$ (1) = 1.10), resulting in correlations of –0.135 for MZ and –0.25 for DZ. In the next step, the MZ and DZ r_p were equated, which again resulted in a non-significant decline in fit ($\Delta\chi^2$ (1) = 1.37) indicating that the difference between the MZ r_p and DZ r_p is not significant (which was expected under the biometrical model used for the analysis).

Second, the r_{xt} is higher among MZ pairs (mean, $r = -0.18$) than among DZ pairs (mean, $r = -0.03$), suggesting that genetic factors play a role in the observed association between CP and CA. If the entire association is due to genes, r_{xt} in MZ twins should be the same as r_p in MZ pairs. If, on the other hand, r_p is higher than r_{xt}, this would suggest that individual environmental factors contribute to the association. The results suggested this was the case (mean r_p, $r = -0.22$ > mean r_{xt}, $r = -0.18$), although the difference was small. Hence, the examination of the 3 kinds of correlations suggests that genetic factors as well as individual-specific factors play a causative role in the association between CP and CA.

Back to outline

Structural Equation Modeling

Univariate

Univariate structural equation modeling of the entire CBCL data (n = 752 pairs) suggested a best-fitting model with a genetic factor explaining 41% of the variance (95% CI: 31%-53%), a common environmental factor explaining 42% of the variance (95% CI: 31%-52%) and an individual-specific environmental factor explaining 17% of the variance (95% CI: 14%-19%). Univariate model fitting on the whole IQ data set (n = 663 pairs) resulted in a best-fitting model with a genetic component explaining 83% of the variance (95% CI: 79%-86%) and an individual-specific factor explaining 17% of the variance (95% CI: 14%-21%).

Bivariate

Bivariate model fitting began with the full model (model 1 in table 3), allowing for additive genes (A), common environment (C), and individual-specific environment (E) for both CP and CA and allowing for genetic, common environmental and individual-specific environmental correlations between them: r_a, r_c and r_e respectively.

Based on the results of the univariate model fitting and the correlation analysis, we changed the full model by omitting the common environmental factor in CA and the common environmental correlation between CP and CA. This model fitted nearly as well as the first one ($\chi^2_{\text{model 2}} - \chi^2_{\text{model 1}} = 2.53$, $\Delta df=2$, $p>0.05$) and was more parsimonious.

Based on model 2, we fitted a third model in which the genetic correlation between CP and CA was set to 0, forcing the model to explain all of the association between CP and CA by means of individual-specific factors. However, the fit of this model was substantially worse than that of model 2. We thus fitted a fourth model, also based on the second model, now forcing the model to explain all of the association by means of genetic factors (the individual-specific correlation was set to 0). The fit of this model also was worse than model 2.

Therefore, model 2 remained the best fitting model statistically and was also the most plausible, taking the results of the univariate model fitting and those of the correlation analysis as template.

The parameter estimates for the model with the best fit are given in table 4 and illustrated in Figure 2. The genetic correlation (r_a) between CP and CA was estimated at –0.27 (95% CI: -0.42; -0.12) (the negative correlation indicating that genetic factors causing an increase on the CBCL were associated with the genetic factors causing a lower WISC-R

Back to outline

score). So, the bivariate heritability equalled: $\sqrt{a^2_{cbcl}} \times r_a \times \sqrt{a^2_{wisc-r}} = \sqrt{0.41} \times (-0.27) \times \sqrt{0.83}$ =-0.16 The average phenotypic correlation between CP and CA equalled - 0.19 (95% CI:-0.09,-0.27). Thus, 84% of the phenotypic correlation was due to shared genetic effects (- 0.16/-0.19 =0.84).

The individual-specific correlation (r_e) between CP and CA equalled –0.17 (95% CI: -0.31; -0.03). Therefore, the bivariate e^2: $\sqrt{e^2_{cbcl}} \times r_e \times \sqrt{e^2_{wisc-r}} = \sqrt{0.17} \times (-0.27) \times \sqrt{0.17} = -0.03$.

The average phenotypic correlation between CP and CA equalled - 0.19 (95% CI:-0.09,-0.27). Thus, 16% of the phenotypic correlation was due to individual specific effects (- 0.03/ -0.19 =0.16).

DISCUSSION

Genetic factors accounted for 84% of the observed phenotypic correlation. However, the observed phenotypic correlation itself was small (average correlation: -0.19). These modest correlations are similar to those reported in pervious studies (30) and are commonly found in general population samples. So, this means that the total variance in CP explained by genetic factors relating to CA (and vice versa) is small.

Our analyses indicate that in children three different genetic factors exist that (1) solely influence the liability for CP, (2) only affect CA and (3) influence both CP and CA. Regarding environmental effects, our results indicate that there are individual-specific environmental factors that influence the vulnerability to both traits, although most of the environmental factors that increase the risk of CP do not influence CA and vice versa.

These conclusions should be interpreted in the context of seven limitations. First, an epidemiological twin design was used. Epidemiological studies are free from the sorts of referral biases that complicate the interpretation of clinical studies. They habitually collect their data from large and representative samples, leading to generalisable conclusions. However, they often lack the diagnostic expertise of symptom or syndrome recognition. Another disadvantage is that even large epidemiological studies include relatively small numbers of subjects with the specific trait/disorder that the study focuses on, resulting in low statistical power. Second, this paper examined the covariance between individual differences in intelligence and psychopathology in a normal population of young twins. In fact, the

Back to outline

association between low CA (low WISC-R scores) and behavioural problems (high CBCL-score) was not analysed. One might hypothese that the mechanisms causing CP in individuals with low IQ (and vice versa) might be different from those in individuals with IQ and CBCL within the normal range. Third, reviews of previous twin studies (16) show that the relative effect of genes on CA becomes greater with age and that at the same time the common environmental effects decrease. Knowing that the estimate of the genetic correlation between two traits is influenced by the estimate of the genetic influence on each trait separately, it is possible that, if the genetic influence on two traits is age-dependent, the estimate of the genetic correlation between those two traits is also age-dependent. The reported genetic correlation of –0.27 should therefore be seen as an estimate of the genetic correlation between CP and CA for children aged 8-14 years only. Fourth, although previous work suggests that estimates of the genetic contribution to variation in cognitive ability do not differ significantly between boys and girls (31), there may be an influence of sex on the estimate of the genetic influence on CA. It is worthwhile examining whether the estimate of the genetic correlation is different for boys or girls. However, a larger sample size would be needed. Fifth, causal models that assume that the association between CP and CA arises by CP directly influencing CA or vice versa, were not described in this paper. Heat et al (32) have shown that there is little or no power in cross-sectional twin data to discriminate the different causal models (reciprocal and two or uni-directional). Only when the two variables have a different genetic structure and the sample size is reasonably large, causal models can be tested. Simonoff (33) has also shown that very large sample sizes are needed to discriminate between the different models. We did post-hoc tests of causality using structural equation modeling, but, as expected, there was no power to discriminate significantly between the models. It has been suggested that if a direct causal relationship between CP and CA existed, it would be one from CA to CP (30). An alternative, non-statistical, way to gather information about the existence of causality is to evaluate the ages of onset of the pathological extremes of the two traits (high pyschopathology and lower cognitive ability) in comorbid cases. But then arises the question whether lower CA, measured at a given time, should be considered as a stable trait and thus representative for earlier/later CA or as a time-dependent trait and consequently not representative for earlier/later CA. Sixth, CA and CP were measured at two different times with an interval of more than 2.5 years. The advantage of this design is that i) they were assessed by different researchers who were blind for the earlier measurement of CA or CP, leading to two independent measurements, and ii) there is

Back to outline

less risk of test contamination, ie CP influencing the test results of CA or vice versa. A disadvantage, however, may be that in as much as the traits are expressed differentially under the influence of time, their associations will be weakened. Finally, bias testing suggested that the pairs included in the study were slightly different from the register and mother samples in terms of birth weight and IQ, whilst CBCL scores did not differ. Two biases might have arisen as a result. The direction of bias was towards exclusion of children with lower IQ scores but normal CBCL scores. This could have led to an inflated estimate of the association between CA and CP. However, given that the differences between excluded and included groups were very small, it is unlikely that this would have had a major impact on the results, and the consistency of the association between CA and CP has been established across a range of independent studies cited earlier. The second bias is that preferential inclusion of children with higher birth weight could have biased the results if the association between CBCL and IQ varies as a function of birth weight. In order to examine this, a regression analysis was performed of CBCL scores on IQ score, birth weight (corrected for sex and gestational age) and their interaction. The coefficient for the interaction term was neither large (< 0.01) nor significant ($p > 0.5$), indicating that the association between CBCL scores and IQ scores was not modified by birth weight, thus making it unlikely that such a bias was operating.

In conclusion, our results confirmed the existence of an association between child psychopathology and lower cognitive ability. It may be useful for mental health professionals to be aware of the fact that a child with lower cognitive ability has a higher risk, regardless of the direction of causality, for psychopathology and vice versa. Given the existence of diagnostic overshadowing (i.e. the tendency to minimize or misdiagnose psychiatric disorders in the presence of low cognitive ability) (34), this should be a special point of attention. Early identification of intellectual deficits among preschoolers may help to prevent later school difficulties and severe psychopathology (1).

The largest part of the observed association between CP and CA is explained by genetic factors. The knowledge that there may be genes that influence CP as well as CA can help the search for specific genes in comorbid samples. The estimated parameters in the bivariate model can be used to provide estimates of individual genetic and environmental factor scores (35). Boomsma (36) has shown that the use of such factor scores increases the power to detect a quantitative trait locus (QTL). Although only a small part of the association

Back to outline

between CP and CA is caused by individual-specific environmental factors, it may be worthwhile to identify these and examine the degree to which they can be modified.

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Back to outline

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Back to outline

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Back to outline

Table 1: Bias testing between included and excluded subjects

		Include			Excluded			χ^2 (df)	t (df)
		N	*%*	*Mean (SD)*	*N*	*%*	*Mean (SD)*		
Gender[1]	Male	354[a]	47.07		946[a]	49.6		1.47 (1)	
	Female	398[a]	52.93		958[a]	50.3			
Birthweight (gram)[1]		752[a]		2507.05	1895[a]		2428.50		3.65 (1558.5)***
Zygosity[1]	MZ	168[b]	44.68		359[b]	38.3		4.53 (1)*	
	DZ	208[b]	55.32		578[b]	61.6			
Fertilization[1]	Spontaneous	291[b]	80.61		757[b]	82.0		0.34 (1)	
	Induction	70[b]	19.39		166[b]	17.9			
Gestation (weeks)[1]		372[b]		36.81 (2.33)	791[b]		36.59 (2.77)		1.39 (852.6)
Mother age (years)[1]		368[b]		27.33 (4.04)	929[b]		27.07 (4.38)		0.97 (1295.0)
Father age (years)[1]		273[b]		29.13 (4.31)	692[b]		29.32 (5.13)		-0.60 (589.0)
CBCL[2]		752[a]		3.99 (1.64)	749[a]		3.83 (1.87)		-1.78 (1472.8)
WISC-R[3]		752[a]		104.22	546[a]		99.93 (15.14)		-5.20 (1296.0)***

[1] included subjects compared with all subjects registered in the EFPTS born alive between 1980 and 1989
[2] included subjects compared with the non-overlapping subjects from the original CBCL study
[3] included subjects compared with the non-overlapping subjects from the original IQ study
[a] number of children, [b] number of twin pairs.
* $p < 0.05$, *** $p < 0.001$

Back to outline

Table 2: Correlations between child psychopathology and cognitive ability in MZ and DZ twinpairs[a]

	T1 CBCL	*T1 WISC-R*	*T2 CBCL*	*T2 WISC-R*
T1 CBCL	-	-0.2058 **	0.79***	-0.1983**
T1 WISC-R	-0.123	-	-0.1707*	0.8045***
T2 CBCL	0.5733***	-0.0436	-	-0.2392**
T2 WISC-R	-0.016	0.5327***	-0.1604*	-

[a] Correlations in MZ twin (168 pairs) are above the diagonal, those for DZ twin (208 pairs) are below the diagonal. CP was assessed by the CBCL, CA was assessed by the WISC-R.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Back to outline

Table 3 : Results of Bivariate Model Fitting[a]

	Model 1	Model 2 ‡	*Model 3*	*Model 4*
CBCL	ACE	ACE	ACE	ACE
WISC-R	ACE	AE	AE	AE
r_a	F	F	0	F
r_c	F	-	-	-
r_e	F	F	F	0
- 2 LnL	9662,27	9664,80	9677,37	9670,27
Df	2790	2792	2793	2793

[a] CP was assessed by CBCL, CA was assessed by WISC-R.
A indicates additive genetic factors; C, common environmental factors; E, individual-specific environmental factors; r_a, genetic correlation; r_c, common environmental correlation; r_e, individual-specific environmental correlation; F, free (parameter free to take any value); 0, parameter fixed to 0; ‡, best fitting model.

Back to outline

Table 4: Parameter estimates and the 95% confidence intervals for the bivariate models for CP and CA [a]

CBCL	*WISC-R*	r_a (95% CI)	r_c (95% CI)	r_e (95% CI)
ACE	ACE	-0.33 (-0.54, -0.09)	0.12 (-1,1)	-0.16 (-0.30, - 0.01)
ACE ‡	AE ‡	-0.27 (-0.42,-0.12)	-	-0.17 (-0.31, -0.03)
ACE	AE	0	-	-0.25 (0.37,- 0.12)
ACE	AE	-0.33 (0.47, -0.19)	-	0

[a] CP was assessed by CBCL, CA was assessed by WISC-R.
r_a indicates genetic correlation; r_c, common environmental correlation; r_e individual-specific environmental correlation
‡ best fitting model

Back to outline

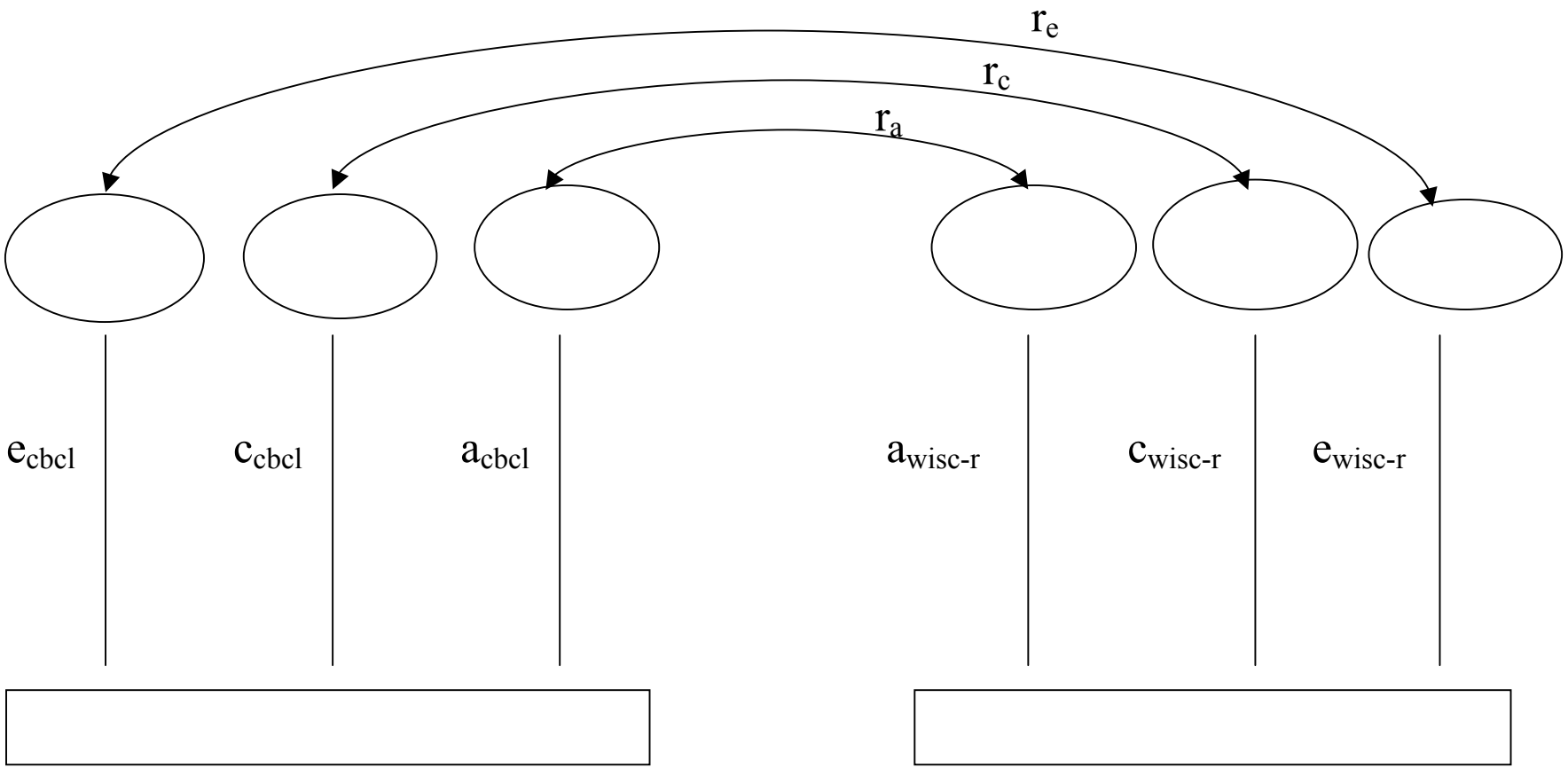

Figure 1 A full bivariate twin model for CP, measured by CBCL, and CA, measured by WISC-R. The variance in liability to each trait is divided into that due to additive genetic factors (A_{cbcl} and A_{wisc-r}), common environmental effects (C_{cbcl} and C_{wisc-r}) and individual-specific environmental factors (E_{cbcl} and E_{wisc-r}). Paths, which are the standardized regression coefficients, must be squared to equal the proportion of variance accounted for. They are represented by lowercase (a, c and e) with the subscripts cbcl and wisc-r. The phenotypic correlation between CP and CA is, in this model, decomposed into that due to the correlation of additive genes (r_a), and the correlation of common environmental factors (r_c) and the correlation of individual-specific environmental factors (r_e).

Back to outline

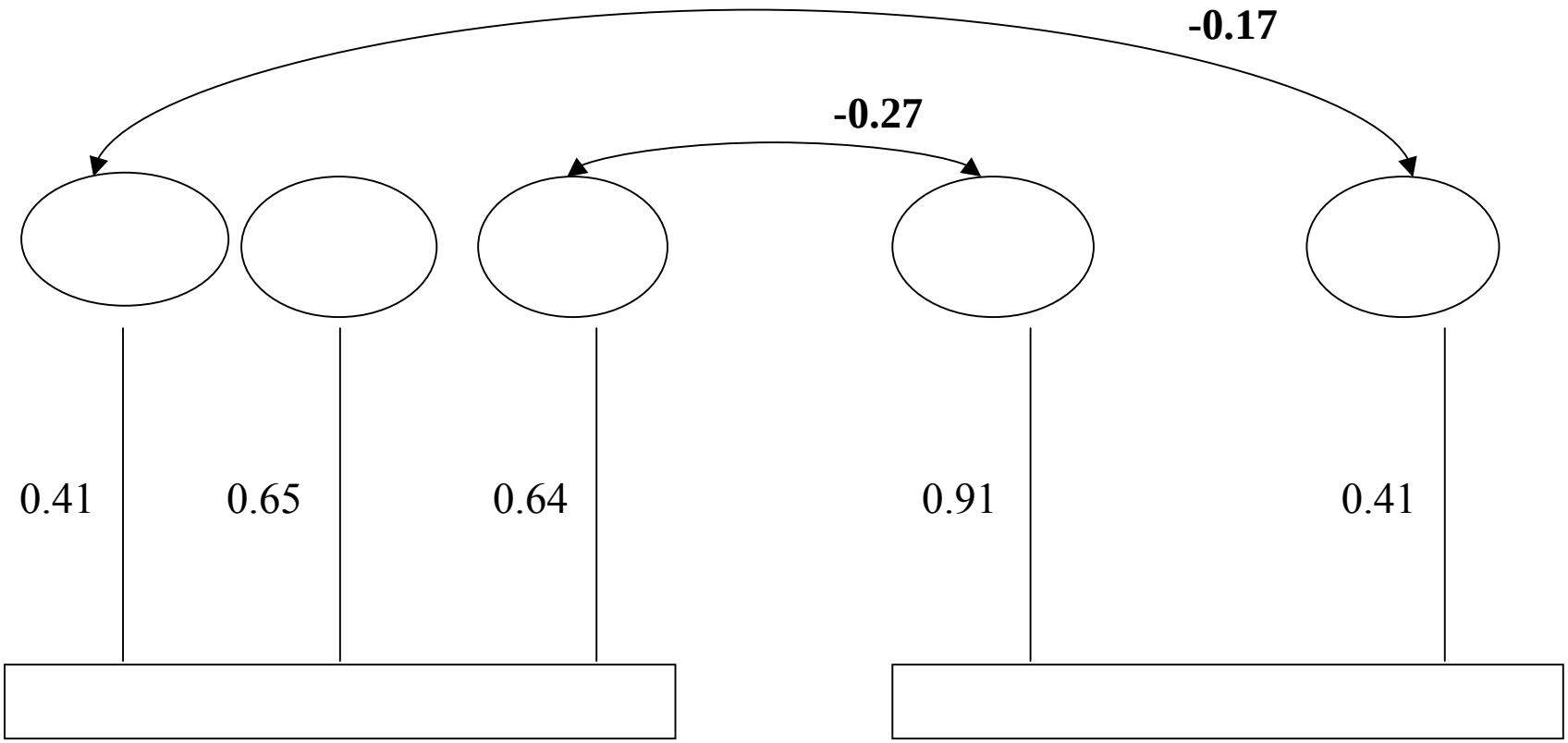

Figure 2 The parameter estimates from the best fitting bivariate model (model 2, table 2) for CP, measured by CBCL, and CA, measured by WISC-R. Path coefficients must be squared to equal the proportion of variance accounted for in the dependent variable. A_{cbcl},C_{cbcl}, A_{cbcl}, $A_{wisc\text{-}r}$, $E_{wisc\text{-}r}$ are explained in the legend to Figure 1.

Back to outline

Chapter 5:

Deconstructing the familiality of the emotive component of psychotic experiences in the general population

Nele Jacobs, Inez Myin-Germeys, Catherine Derom, Robert Vlietinck and Jim van Os

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Back to outline

ABSTRACT

Twin studies of the subclinical expression of positive and negative features of psychosis typically focuses on the occurrence of subclinical psychosis *per se*, whereas the risk of subsequent development of clinical need may be more related to the tendency to develop associated distress. Genetic influences on variation in distress associated with psychotic experiences was examined in a twin study.

289 Belgian twin pairs filled in the Community Assessment of Psychic Experiences (CAPE), a 42-item self-report instrument assessing subclinical positive and negative psychotic experiences and associated distress ($distress_{pos}$ and $distress_{neg}$). Using structural equation modeling, univariate and bivariate models were fitted. The best fitting models (based on likelihood and parsimony) were chosen.

Univariate model fitting showed genetic and non-shared environmental influences on both $distress_{pos}$ and $distress_{neg}$. Bivariate model fitting showed that 52% of the correlation between the two distress phenotypes (r= 0.46) was due to shared genes and that non-shared environmental factors accounted for 48% of the correlation.

Liability to psychosis not only refers to the development of psychosis per se, but also to the liability to develop dysfunctional emotional appraisals. The emotive component of psychosis liability involves genetic transmission of a general, non-symptom specific distress factor that may be a plausible target for molecular genetic research.

Key words: distress, psychotic symptoms, general population, twin study, model fitting

Back to outline

BACKGROUND

Subclinical psychotic experiences, or "schizotypy", are the phenotypic expression of the familial liability to schizophrenia. Thus, relatives of patients with schizophrenia score significantly higher on measures of schizotypy (1-3). In addition, twin studies have quantified the genetic liability of schizotypy and most reported moderate genetic effects for measures of general schizotypal traits, for example assessed with the Schizotypal Personality Scale (4, 5). However, studies have shown that schizotypy is a multidimensional construct consisting of at least three factors of which two have been most consistently replicated: i) positive psychotic experiences and ii) negative psychotic experiences (6-8). Twin studies have shown that genetic factors also play a role in these specific dimensions of schizotypy (9-14).

All previous work has focused on the occurrence and frequency of subclinical psychotic experiences in the general population. However, it has been suggested that it is not only the frequency or intensity of subclinical psychotic experiences but also the associated distress that is important in the development of clinical need and patient status. (15-21).Therefore, it seems crucial to additionally study the genetic liability of the tendency to experience distress related to subclinical psychotic experiences, if the pattern of familial transmission of psychotic disorders is to be fully understood.

A number of recent studies (22, 23) investigated patterns of familial transmission of psychological distress in general population samples. A moderate genetic effect has been found for general psychological distress in an adult general population sample (44% of the phenotypic variation explained by additive genetic effects) and in pre-school children (additive genetic effects explaining 43% of the phenotypic variation). The latter study (23) also reported moderate genetic overlap between general distress, anxiety and fatigue, suggestive of a more general underlying distress factor.

Aims of the study

The current study investigated the familiality of distress associated with positive and negative subclinical psychotic experiences in a general population twin-sample. Furthermore, it was studied whether distress associated with positive psychotic experiences and distress associated with negative psychotic experiences, was specific to the symptom dimension or represented a general underlying distress factor.

Back to outline

METHODS

Sample

The study sample consisted of 177 monozygotic and 112 dizygotic female twin pairs between 18 and 46 years of age from Flanders (Belgium). Two-hundred and twenty-nine pairs came from the East Flanders Prospective Twin Survey. This population-based survey has prospectively recorded all multiple births in the province of East Flanders since 1964 (24, 25). Perinatal data were collected at birth, and placental examination was performed within 48 hours after delivery. Zygosity was determined through sequential analysis based on sex, fetal membranes, blood groups and DNA fingerprints. Sixty pairs were recruited using registers from Flemish municipalities. Determination of zygosity in these twins was based on their and their mothers' response to standard questions about physical similarity and the degree to which others confused them (26-28) and if necessary, on examination of DNA fingerprints. The project was approved by the Local Committee of Medical Ethics and all participants gave written informed consent

Measures

Subclinical psychotic experiences were assessed with the Community Assessment of Psychic Experiences (29); http://www.cape42.homestead.com/). The CAPE is a self-report questionnaire, developed in order to rate self-reports of attenuated psychotic experiences in the affective and non-affective domains. The CAPE measures, on a dimensional scale, frequency as well as distress associated with these experiences. The frequency score is measured on a 4-point scale from "never [1]", "sometimes [2]", "often [3]" to "nearly always [4]". The degree of distress associated with the experience is also measured on a 4-point scale ranging from "not distressed [1]", "a bit distressed [2]", "quite distressed [3]" to "very distressed [4]". The CAPE includes psychosis dimensions of positive, negative and depressive experiences. The depressive factor was added so as to allow variation of the negative dimension independent of affective symptoms, and because variation in the affective domain can be considered itself as an independent dimension of psychosis (29). Previous research with the CAPE has shown i) a three-factor structure of positive, negative and depressive factors in a large and representative sample of young men (29) and in a large sample of undergraduate female students (30), and ii) discriminative validity across groups of

Back to outline

individuals with schizophrenia, affective and anxiety disorders and individuals from the general population (19). The CAPE positive, negative and depressive symptom dimensions encompass 20, 14 and 8 items, respectively.

The CAPE provides two scores per dimension by i) adding up the scores on the frequency question, yielding a total frequency score, and ii) adding up the scores on the distress questions, yielding a distress score. For each frequency and for each distress score, a weighted score was calculated (sum of frequency/distress questions divided by number of items filled in – minimum score: 1).

Analysis

Univariate analysis

First, intrapair correlations for both MZ and DZ twins were calculated for distress associated with positive symptoms ($distress_{pos}$) and for distress associated with negative symptoms ($distress_{neg}$). Comparison between MZ and DZ intrapair correlations gives a first impression about the role of genetic and environmental factors. If the MZ intrapair correlation is about twice as high as the DZ intrapair correlation, additive genetic factors (A; the sum of the average effects of the individual alleles at all loci affecting the phenotype) and individual-specific factors (E; environmental influences that are not shared between family members) are likely to play a role in determining individual differences in the trait. If, on the other hand, the MZ intrapair correlation is lower than two times the DZ intrapair correlation, additive genetic factors (A), common environmental factors (C; environmental influences shared between family members) and individual-specific factors (E) are assumed to play a role.

The results of the intrapair correlation analysis guided the univariate structural equation modeling, using the Mx programme (31). Univariate structural equation modeling decomposes the phenotypic variance into genetic and environmental factors (see above) and estimates the genetic and environmental parameters. Different models were fitted to the data and compared using difference in fits and difference in degrees of freedom as criterion (32). The best fitting model was chosen based on likelihood and parsimony of the model. The 95% confidence intervals were calculated (33).

Bivariate analysis

The causes of association between two variables can usually be intuited from the pattern of the bivariate correlations. If the MZ cross-twin cross-variable correlation (i.e. $distress_{pos}$ in twin 1 correlated with $distress_{neg}$ in twin 2 and vice versa) is significantly higher than the DZ

Back to outline

cross-twin cross-variable correlation, a genetic factor can be hypothesized to play a role in the association between the two phenotypes. In addition, if all of the association is due to genes, then the MZ within-twin cross-variable correlation (i.e. $distress_{pos}$ in twin 1 correlated with $distress_{neg}$ in twin 1 and same for twin 2) should be the same as the MZ cross-twin cross-variable correlation. If, on the other hand, the MZ within-twin cross-variable correlation is higher than the MZ cross-twin cross-variable correlation, individual-specific environmental factors are also likely to contribute to the association. The results of the correlation analysis, together with the results of the univariate analysis, were used as a guide for the bivariate structural equation modeling, using Mx (31). The goal of a bivariate structural equation modeling is to decompose the covariance between two associated phenotypes into genetic and environmental factors (see above) and to estimate the genetic and environmental correlations. Different models were fitted to the data and compared using difference in fits and difference in degrees of freedom as criterion (32). The best fitting model was chosen based on likelihood and parsimony of the model. The 95% confidence intervals were calculated (33). Next, the bivariate heritability (that part of the phenotypic correlation due to shared genes: $\sqrt{a^2}_{distress\ pos} \times r_a \times \sqrt{a^2}_{distress\ neg,}$ and/or the bivariate c^2 (that part of the phenotypic correlation due to shared common environmental factors: $\sqrt{c^2}_{distress\ pos} \times r_c \times \sqrt{c^2}_{distress\ neg}$) and the bivariate e^2 (that part of the phenotypic correlation due to shared individual-specific environmental factors: $\sqrt{e^2}_{distress\ pos} \times r_e \times \sqrt{e^2}_{distress\ neg}$) were calculated.

RESULTS

Sample

289 female twin pairs from Flanders (Belgium) participated: 177 monozygotic and 112 dizygotic. Mean age of the sample was 27 years (7.5 years SD, range 18-46 years). 60.8% had a college or university degree, 36.9% completed secondary education and 2.3% had a primary education. The majority was currently employed (63.4% employed, 31.7% student, 2.1% unemployed, 2.3% homemaker and 0.5% sick leave). Mean weighted frequency of positive psychotic symptoms per subject was 1.2 (0.18 SD, range 1-2.1), of negative psychotic symptoms 1.5 (0.3 SD, range 1-2.9) and of depressive symptom was 1.7 (0.4 SD, range 1-3.6). Weighted distress scores associated with positive and negative symptoms were calculated for subjects with at least one positive, respectively one negative symptom (score of "sometimes" or more). The distribution of $distress_{pos}$ was skewed, $distress_{neg}$ was distributed

Back to outline

normally. The mean of $distress_{pos}$ was 1.7 (n=512, 0.5 SD, range 1-4; median=1.7) and of $distress_{neg}$ was 1.9 (n=547, 0.5 SD, rang 1-3.8).

Univariate analysis

The MZ intrapair correlation for $distress_{pos}$ equalled 0.35 ($p < 0.05$), while the DZ intrapair correlation for $distress_{pos}$ equalled –0.04 ($p > 0.05$) (Table1). Genetic factors as well as individual-specific factors were hypothesized to play a role. Univariate structural equation modeling confirmed this assumption (Table 2). The best fitting model was the model with a genetic factor, explaining 32% of the variance (95% CI:0.17-0.46) and an individual-specific environmental factor, explaining 68% of the variance (95% CI:0.54-0.83).

The MZ intrapair correlation for $distress_{neg}$ was 0.43 ($p < 0.05$), the DZ intrapair correlation was 0.02 ($p > 0.05$) (Table 1), suggesting genetic and individual-specific environmental influences. Univariate structural equation modeling (Table 2) again confirmed this, showing a best fitting model with a genetic factor, explaining 41% of the variance (95% CI:0.28-0.53) and an individual-specific environmental factor, explaining 59% of the variance (95% CI:0.47-0.72).

Bivariate analysis

Correlations (Table 1)

The average correlation between $distress_{pos}$ and $distress_{neg}$ equalled 0.46 (95% CI: 0.38-0.53). The MZ cross-twin cross-variable (mean, r=0.23) is higher than the DZ cross-twin cross-variable correlation (mean, r = -0.02), suggesting that genetic factors play a role in the observed phenotypic correlation. The within-twin cross-variable correlation in MZ twins (r = 0.43) was higher than the MZ cross-twin cross-variable correlation, suggesting that individual-specific environmental factors also contribute to the phenotypic correlation. Hence, examination of the correlation suggests that genetic factors as well as individual-specific environmental factors play a causative role in the correlation between $distress_{pos}$ and $distress_{neg}$.

Structural equation modeling

Bivariate modeling fitting began with the full model (model 1 in table 3), allowing for additive genes (A), shared environmental genes (C) and individual-specific environmental genes (E) for both $distress_{pos}$ and $distress_{neg}$ and allowing for additive genetic, shared

Back to outline

environmental and individual-specific environmental correlations between them: r_a, r_c and r_e respectively.

Based on the results of the univariate model fitting and the correlation analysis, the full model was changed omitting the shared environmental factor in $distress_{pos}$ and $distress_{neg}$ and consequently also the shared environmental correlation between $distress_{pos}$ and $distress_{neg}$. This model fitted as well as the full model and was more parsimonious ($\chi^2_{model\ 2} - \chi^2_{model\ 1} = 0$, $\Delta df=3$, $p=1$). Based on model 2, a third model was tested in which the genetic correlation between $distress_{pos}$ and $distress_{neg}$ was set to 0, forcing the model to explain all of the association by means of individual-specific environmental factors. However, the fit of this third model was significantly worse than model 2 ($\chi^2_{model\ 3} - \chi^2_{model\ 2} = 16.75$, $\Delta df=1$, $p<0.01$). A fourth model, also based on the second model, was tested, now forcing to explain all of the association by means of genetic factors (the individual-specific correlation was set to 0). The fit of this model was also worse as the fit of the second model ($\chi^2_{model\ 4} - \chi^2_{model\ 2} = 20.94$, $\Delta df=1$, $p<0.01$). Therefore, model 2 remained the best fitting model statistically and was also the most plausible, taking the results of the univariate model fitting and the correlation analysis as a template.

The parameter estimates for the best fitting model are illustrated in Figure 1. The correlation between the genetic factors in both $distress_{pos}$ and $distress_{neg}$ was estimated at 0.66 (95% CI:0.42-0.86). Therefore, the bivariate heritability equalled: $\sqrt{a^2}_{distress\ pos} \times r_a \times \sqrt{a^2}_{distress\ neg} = \sqrt{0.32} \times 0.66 \times \sqrt{0.41} = 0.24$. The average phenotypic correlation equalled 0.46 (95% CI: 0.38-0.53). Thus, 52% of the phenotypic correlation between $distress_{pos}$ and $distress_{neg}$ was due to shared genetic effects (0.24/0.46=52.2).

The correlation between the individual-specific environmental factors in both $distress_{pos}$ and $distress_{neg}$ was estimated at 0.35 (95%CI: 0.20-0.48) and the bivariate e^2 equalled: $\sqrt{e^2}_{distress\ pos} \times r_e \times \sqrt{e^2}_{distress\ neg} = \sqrt{0.68} \times 0.35 \times \sqrt{0.59} = 0.22$. The average phenotypic correlation equalled 0.46 (95% CI: 0.38-0.53), indicating that 48% of the phenotypic correlation between $distress_{pos}$ and $distress_{neg}$ was due to individual-specific environmental factors (0.22/0.46=47.8).

DISCUSSION

This was the first study ever to focus on the familiality of distress associated with subclinical psychotic experiences in the general population. The results of the univariate analysis showed that both distress associated with positive psychotic experiences and distress associated with

Back to outline

negative psychotic experiences were influenced to a moderate degree by a genetic factor. In addition, the results of the bivariate analysis demonstrated that the correlation between the two phenotypes was explained by shared genetic and environmental factors, suggestive of a general, non-symptom specific distress factor.

The results are important in view of previous research suggesting that level of distress associated with psychotic-like symptoms, more than frequency or intensity of these symptoms, is related to clinical need and patient status (15-21). Several studies already demonstrated that subclinical psychotic experiences are much more prevalent in the general population than psychotic disorders found in clinical settings (30, 34-36). It has been suggested that feelings of distress are crucial in the development of patient status. This also fits with data showing that neuroticism, a tendency to feel distressed and experience negative emotions, significantly increases the risk to develop psychotic disorders (37, 38). The current study suggests that more than 50% of the variation in feelings of distress in the context of psychosis, and thus the risk of developing need for care, is due to genes.

The results of the bivariate analysis demonstrated that the correlation between distress associated with positive psychotic experiences and distress associated with negative psychotic experiences is explained by shared genetic and environmental factors. These results, therefore, are suggestive for the existence of a general, non-symptom specific distress factor. Thus, the risk of becoming a patient may in part have its substrate in the individual genetic vulnerability to feel distressed in the face of psychotic experiences. Given these results, it is attractive to hypothesize that the nature of the psychotic experience (positive, negative, depressive) may determine what type of psychopathology is developed, while the risk of actually developing need for care is, amongst others, based on the vulnerability to feel distressed by early psychopathological experiences.

As this study suggested the existence of a general, non-symptom specific distress factor, which may determine the risk of becoming a psychiatric patient, it may be worthwhile to identify factors influencing this general distress factor. The results of the bivariate analysis showed influences of both genetic and individual-specific environmental factors. Social support may be an example of such an individual-specific environmental factor. It is well known that lack of social support is associated with increased risk of psychiatric disorder (39, 40). When experiencing a psychiatric symptom, lack of social support may increase the distress associated with that symptom, resulting in a higher risk of developing need for care. Much less is known about genetic factors influencing distress. A possible candidate is a

Back to outline

functional polymorfism on the serotonine transporter gene (SERT). It has been demonstrated that SERT sites in the human brain are associated with emotion functions (41). In addition, there is some evidence that SERT might be involved in psychotic disorders (42) and the tendency to develop depression after exposure to stress (43)**.** The results of the current study may aid in identifying more effects associated with these genes.

This study explored the familiality of distress associated with subclinical psychotic experiences in the general population. Experiencing sub-clinical psychotic symptoms and reporting associated distress are seen as possible risk factors for actually developing a psychotic disorder. The main age range of risk for psychotic disorders is between 20 to 35 years and on average, women fall ill 3 to 4 years later than men and show a second peak of onset around menopause. Therefore, the age range in this sample (18 to 46 year) was appropriate to detect the occurrence of subclinical psychotic experiences and the distress associated with it.

These results should be viewed in the light of several methodological issues.
First, as this sample consisted of only female adults, the results of this study consequently apply to female populations only. Simultaneous analysis of male and female MZ and DZ pairs as well as opposite sex pairs will provide an opportunity to study sex differences in distress associated with psychotic symptoms. Second, more than 60% of the sample consisted of people with a higher educational degree, affecting representativeness in respect to the general population. If this non-representativeness would bias the data, then it would probably influence the prevalence of psychotic symptoms and the associated distress. However, this paper did not focus on the prevalence of psychotic symptoms and associated distress, but investigated the role of genetic and environmental factors in distress related to psychotic experiences. There is no reason to assume that the impact of genetic and environmental factors on distress would differ between lower and higher educated people. Therefore, it is very unlikely that the results of the genetic analysis would be biased by the non-representativeness of the sample.
Third, it is conceivable that other selections might be operating, with the risk of bias such as self-selection, a defensive responding style or ethnic selection. However, for any of these factors to result in bias, they would be required to be associated with genetic factors, which is not likely. If these factors played any role, it would most likely be in the realm of underreporting of psychotic experiences and associated distress and, by consequence, in

Back to outline

reduced statistical power. Nevertheless, the results of the statistical analysis in this study are robust and it is plausible to assume that the results of this study would be even more robust, rather than qualitatively different, in the case of increased statistical power.

Fourth, the MZ intrapair correlation for both $distress_{pos}$ and $distress_{neg}$ is much higher than two times the DZ intrapair correlation. This pattern suggests that both phenoypes also are influenced by non-additive genetic effects. However, our sample size of 289 twin pairs is far too small to detect non-additivity (3). More precisely, the power to identify a full ADE-model (with A explaining 30% of the variation and D explaining 30% of the variation) in both $distress_{pos}$ and $distress_{neg}$, is about 12%. A sample size of at least 3000 twin pairs would therefore be needed. In addition, discrimination between different sources of non-addivity (dominance, epistasis and sibling interaction) is not possible in this sample. A much lager twin sample, extended with data on parents, children, siblings and spouses, is needed to allow reliable discrimination between the alternative genetic sources (3). However, the DZ intrapair correlation for both phenotypes was around 0 in this sample. This pattern, in combination with MZ intrapair correlations that were much higher than two times the DZ intrapair correlations, is suggestive of epistasis or interaction between alleles at different chromosomal loci. Unfortunately, this hypothesis could not be verified given the limited statistical resolution.

Nevertheless, even though this study lacked power to distinguish between additive and non-additive genetic effects, it clearly showed that genetic effects have a substantial impact on both phenotypes as well as on the correlation between them.

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Back to outline

Table 1: Correlations between distress associated with positive psychotic symptoms ($distress_{pos}$) and distress associated with negative psychotic symptoms ($distress_{neg}$) in MZ and DZ twinpairs[a].

	t1 $distress_{neg}$	t1 $distress_{pos}$	t2 $distress_{neg}$	t2 $distress_{pos}$
t1 $distress_{neg}$	-----	0.51***	0.43***	0.26**
t1 $distress_{pos}$	0.49***	-----	0.21*	0.35***
t2 $distress_{neg}$	0.02	-0.05	-----	0.36***
t2 $distress_{pos}$	0.00	-0.04	0.48***	-----

[a] Correlations in MZ twins are above the diagonal, those for DZ twins are below the diagonal

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Back to outline

Table 2: Results of univariate structural equation model fitting[a]

	Model	X^2	*df*	*p*	*AIC*	*a*	*95% CI a*	*c*	*95% CI c*	*e*	*95%CI e*
$distress_{pos}$	ACE	4.58	3	0.20	-1.42	0.32	0.09-0.46	0	0.-0.16	0.68	0.54-0.83
	AE♦	4.58	4	0.33	-3.42	0.32	0.17-0.46	--	--	0.68	0.54-0.83
	CE	10.57	4	0.03	2.57	--	--	0.2	0.08-0.33	0.79	0.67-0.92
	E	20.33	5	0.001	10.33	--	--	--	--	1	1-1
$distress_{neg}$	ACE	8.19	3	0.04	2.19	0.41	0.23-0.53	0	0-0.14	0.59	0.47-0.72
	AE♦	8.19	4	0.08	0.19	0.41	0.28-0.53	--	--	0.59	0.47-0.72
	CE	19.25	4	0.001	11.25	--	--	0.29	0.17-0.39	0.71	0.60-0.83
	E	41.46	5	0.0	31.47	--	--	--	--	1	1-1

[a] A indicates additive genetic factors; C, common environmental factors and E, individual-specific environmental factors

a indicates the estimate of the additive genetic factor, 95% CI a indicates the 95% confident interval for the estimate for a

d indicates the estimate of the common environmental factor, 95% CI c indicates the 95% confident interval for the estimate for c

e indicates the estimate of the individual-specific environmental factor, 95% CI e indicates the 95% confident interval for the estimate for e

♦ indicates the best fitting model, based on fit and parsimony

Back to outline

Table 3: Results of the bivariate structural equation model fitting [a]

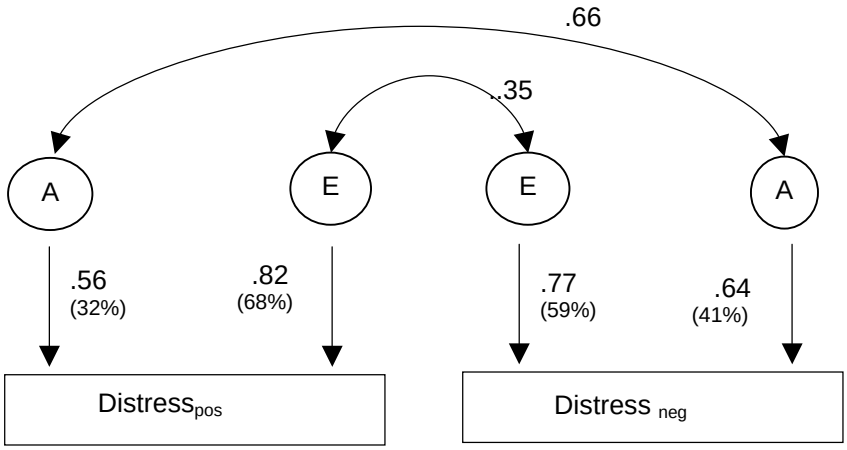
	Model 1	*Model 2* ♦	*Model 3*	*Model 4*
$distress_{pos}$	ACE	AE	AE	AE
$distress_{neg}$	ACE	AE	AE	AE
r_a	F	F	0	F
r_c	F	--	--	--
r_e	F	F	F	0
-2 LnL	1423.30	1423.30	1440.05	1444.24
df	1048	1051	1052	1052

[a] A indicates additive genetic factors; C, common environmental factors; E, individual-specific environmental factors; r_a additive genetic correlation; r_c common environmental correlation and r_e, individual-specific environmental correlation; F, free (parameter free to take any value); 0, parameter fixed to 0.

♦ indicates the best fitting model, based on fit and parsimony

Back to outline

Figure 1: The parameter estimates from the best fitting bivariate model (model 2, Table 3) [a]



[a] Path coefficients must be squared to equal the proportion of variance accounted for in the dependent variable.

Back to outline

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Back to outline

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Back to outline

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Back to outline

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Back to outline

Chapter 6:

Electronic monitoring of salivary cortisol sampling compliance in daily life

Nele Jacobs, Nancy Nicolson, Catherine Derom, Philippe Delespaul, Jim van Os and Inez Myin-Germeys

Life Sciences, 76, 2431-2433

Back to outline

ABSTRACT

Naturalistic research methods have been developed to collect data in the daily environment, providing ecological valid measures. Recent reports suggest, however, that compliance with fixed time sampling protocols may be problematic and can bias results. This study investigated compliance with an intensive, random time sampling protocol for salivary cortisol and effects of non-compliance on cortisol results. Twenty female twin pairs and nineteen of their sisters were instructed to take saliva samples when signaled at ten unpredictable moments on each of five consecutive days. Subjects recorded collection times, unaware that compliance with the sampling protocol was being investigated by means of electronic monitoring devices. Samples taken ≤ 15 min after the signal, according to self-report, were defined as adherent to the protocol. Samples taken ≤ 10 min after the self-reported collection time, according to the monitor, were defined as accurate.
Self-reported adherence to the sampling protocol was 96.4%. Verified compliance was somewhat lower, with 81% of all saliva samples accurately timed. Contrary to previous reports, inclusion of non-compliant samples in the analysis did not distort the cortisol diurnal profile. Intensive, random time sampling appears to have advantages over fixed time sampling for obtaining valid cortisol profiles when researchers do not have devices to monitor compliance. Results indirectly support the validity of momentary self-report data about daily experiences obtained with the same sampling methods.

Key words: salivary cortisol, sampling compliance, electronic monitoring, experience sampling, methods

Back to outline

INTRODUCTION

Naturalistic research methods have been developed to collect data during the flow of everyday life, providing ecological valid measures and allowing researchers to pose questions that cannot be answered in the laboratory or with retrospective methods. Such approaches, known as experience sampling method (ESM) or ecological momentary assessment (EMA), provide the opportunity to relate daily experiences to a variety of outcomes, including physiological changes as well as psychological measures such as mood and symptoms (Affleck et al., 1999; Bolger et al., 2003; deVries, 1992; Reis and Gable, 2000; Scollon et al., 2003; Stone et al., 1999). Recent studies have shown, for example, that activities, stressors, social contexts, and associated emotional states have important effects on heart rate and blood pressure (Kamarck et al., 2002) and on hormone levels (Peeters et al., 2003; Smyth et al., 1998; van Eck et al., 1996).

A possible drawback of many naturalistic research designs is the lack of control over participants' compliance with the protocol, as self-reports are completed and samples taken without the researcher being present. Protocol compliance is crucial for the reliability and validity of the data, especially in the case of physiological parameters known to have a circadian rhythm or to be reactive to environmental stimuli. Ambulatory studies of cortisol are a case in point. Cortisol can be reliably assessed in saliva (Kirschbaum and Hellhammer, 1989; Kirschbaum and Hellhammer, 1994), which has greatly facilitated studies in real-life settings. However, concerns have recently been raised about poor compliance in salivary cortisol studies and the effects of inaccurately timed samples on the results (Broderick et al., 2004; Kudielka et al., 2003; Yehuda et al., 2003).

Non-compliance with ambulatory saliva sampling protocols can take several forms. Firstly, a subject may fail to take the saliva sample at all or forget to record the time, resulting in missing data. Secondly, a sample may be taken at an incorrect time (according to the protocol), but the subject reports the actual collection time accurately. This kind of non-compliance does not necessarily bias the results, because observations with deviant times can easily be excluded from the analysis. Thirdly, non-compliance can take the form of a saliva sample taken at an incorrect time and an inaccurate self-report of the collection time. This form poses a serious threat: the invalid cortisol data cannot be excluded from analysis if the researcher has no way to detect inaccurate self-reports. Inaccurately timed samples introduce error variance and may even bias the results.

Back to outline

Two recent studies used electronic monitoring devices to assess non-compliance with salivary cortisol sampling procedures. In the first (Kudielka et al., 2003) community dwelling volunteers collected saliva samples at six times on a single day: at awakening, 30 minutes later, and at four fixed time points between 11:00 and 22:00. Of 42 subjects, 26% failed at least once to collect a sample within an acceptable time window. The subgroup that was unaware that sampling times were being monitored took 84% of all saliva samples on time; informed subjects did better, with 97% of samples taken on time. Although fewer than 10% of the samples were considered out of range in terms of timing, diurnal cortisol profiles differed significantly in compliant versus non-compliant subjects. The cortisol response to awakening was particularly sensitive to non-compliance: compliant subjects showed a robust increase in cortisol, whereas non-compliant subjects showed only minimal changes. In the second study (Broderick et al., 2004), women with fibromyalgia and healthy controls participated in a 7-day salivary cortisol sampling protocol with 5 samples each day: at awakening, 45 minutes later, and at 16:00, 19:00 and 22:00. In subjects unaware of being monitored, self-reported compliance was 93%, but compliance verified by the monitor cap was only 71%. The authors reported significant within-person differences between compliant versus non-compliant days in the cortisol morning response and a measure of diurnal decline. Together, these two studies provide evidence for inaccuracies in self-reported compliance with ambulatory salivary protocols and suggest that this kind of non-compliance can lead to spurious results.

One factor that might have an important influence on compliance rates is the sampling protocol. Both of the above studies combined event-contingent (awakening response) and fixed time sampling procedures. ESM studies have signal-contingent procedures: subjects complete self-reports and (in this case) collect saliva samples in response to auditory signals received at unpredictable moments throughout the day (Peeters et al., 2003; van Eck et al., 1996). The current study extends previous investigations by assessing compliance and its effects on cortisol measures when saliva samples are obtained at frequent, semi-random intervals.

How seriously inaccurately timed samples will influence the cortisol results depends not only on the frequency of non-compliance, but also on the cortisol outcome measure under investigation and the statistical approach taken. For example, exact timing appears to be crucial for reliable measures of the cortisol response to awakening (Kunz-Ebrecht et al., 2004; Pruessner et al., 1997). To date, however, little is known about the sensitivity of other parameters (e.g., diurnal levels and slopes) to deviations in sample collection times, although these are important outcome measures in neuroendocrine studies (Edwards et al., 2001;

Back to outline

Steptoe et al., 2003; Stone et al., 2001). The current study uses multilevel regression to obtain reliable estimates of cortisol levels and slopes and to model the effect of inaccurately timed samples on cortisol outcomes.

In summary, the main goals of the current study were (1) to determine the accuracy of self-reported saliva collection times in an intensive, signal-contingent time-sampling protocol, and (2) to determine whether inclusion of inaccurately timed samples in the analysis would significantly bias the cortisol results. Because it is reasonable to assume that research participants who take saliva samples when signalled and record sampling times accurately will also complete concurrent pen-and-paper forms accurately, this study also provides indirect evidence about how well subjects comply with intensive ESM sampling procedures in daily life.

MATERIALS AND METHODS

Subjects

This study is part of a longitudinal twin study, in which we assess emotional and hormonal reactions to daily life stressors. The current sample of 59 subjects included 20 female twin pairs and 19 non-twin sisters. Seven of the twin pairs were recruited from the East Flanders Prospective Twin Survey (EFPTS), a twin registry recording multiple births in the province of East Flanders (Belgium) since 1964 (Derom et al., 2002; Loos et al., 1998); an additional thirteen pairs were recruited outside the EFPTS. Of the 19 non-twins, 15 were sisters of EFPTS twins and 4 of non-EFPTS twins. Mean age of the sample was 32 years (range 18-52), 66.1% had completed a college or university degree, and the majority was currently employed (73.2% employed, 16% student, 5.4% homemaker, 5.4% unemployed).

Procedure

A research assistant visited each participant at home to explain the study procedures in detail, including experience sampling (Csikszentmihalyi and Larson, 1987; Delespaul, 1995; deVries, 1992) and saliva sampling methods. To enhance compliance, subjects completed a practice form to confirm they understood the procedure and were given a telephone number they could call in case of questions or problems during the ESM sampling period. Subjects

Back to outline

wore a digital wristwatch programmed to emit a signal ("beep") at an unpredictable moment in each of ten 90-minute time blocks between 7:30 and 22:30, on five consecutive days. Three slightly different sampling schedules were used to insure that subjects living in the same household were not signalled at precisely the same moment. Exact sampling times were varied for each of the five days to decrease predictability. After each beep, subjects completed a short self-report form in a pocket-sized booklet, with open questions and rating scales concerning current thoughts, mood, physical and social context. At the same time, they collected a saliva sample with a cotton swab (Salivette, Sarstedt, Etten-Leur, the Netherlands). Swabs had been transferred by the researchers from their original plastic tubes into a separate 100cc bottle with electronic monitoring cap (eDEM^{TM}, Aardex Ltd., Switzerland) for each day. Participants were told that the eDEM unit was designed to keep the swabs sanitary and to reduce humidity. They were instructed to open the monitor only after receiving a beep signal and to remove only one swab each time. Participants were thus unaware that the monitor would register cap opening times or that compliance with the sampling protocol was being investigated. After saliva collection, subjects were instructed to store the swab in the salivette tube and to record the exact time of collection on the label, on which subject code and date had been pre-printed. Samples were stored in subjects' home freezers until transport to the lab, where uncentrifuged samples were kept at –20° C until analysis.

Cortisol assay

Salivary free cortisol levels were determined in duplicate, using a time-resolved immunoassay with fluorescence detection (Dressendorfer et al., 1992). The lower detection limit of this assay was 0.2 nmol/L; interassay and intra-assay coefficients of variation were less than 10%.

Measures of compliance

Saliva samples were obtained as follows: 1) the wristwatch emitted a beep, 2) the subject opened the monitor, removed a swab, and took a saliva sample, 3) the subject inserted the sample into the salivette tube and recorded the time on the label. During this process, the subject also completed a short ESM form. Adherence to the protocol was defined as a time interval of (-5, +15) minutes between time of the signal and self-reported time of the saliva sampling. This conservative time window was chosen to insure that cortisol measures occurred close in time to ESM measures of concurrent experiences, behavior, and emotions. The percentage of data reportedly collected within this time window is the adherence rate (self-reported compliance).

Back to outline

For all the samples that were adherent with the protocol, the accuracy was assessed. Accuracy of the self-report was defined as the time difference, in minutes, between opening of the monitor (as recorded electronically) and the self-reported time on the tube. An interval of (-2, +10) min between reported and monitored times was considered accurate. An observation of –2 min indicates that the saliva sample was taken two minutes before opening of the monitor, which could occur due to small discrepancies between the internal time settings of the monitor and the watch. An observation of +10 min indicates that the sample was inserted into the salivette tube 10 min after the monitor was opened. We considered a delay of 10 min acceptable, taking into account possible time discrepancies between monitor and wristwatch and the time needed to collect the saliva sample. Such minor deviations in the exact timing of samples are very unlikely to have significant effects on the cortisol outcome measures in this study (overall level and diurnal slope).

Statistical analysis

For testing whether inaccurate samples were distributed uniformly over beeps and days (effects of protocol load), repeated measures ANOVA was performed. In case of significant differences, post-hoc comparisons were done.

To examine the effect of sampling inaccuracy on daily cortisol profiles, we applied the multilevel or hierarchical linear model, using procedure XTREG in STATA (Stata, 1999). The multilevel model is a variant of multiple regression, appropriate for data with a hierarchical structure (Snijders and Bosker, 1999). The current dataset has two levels, with momentary cortisol measures (level 1) nested within subjects (level 2).

Raw cortisol measures were log transformed to normalize their distribution. The variable Time was centered by subtracting the mean time of day for all samples from the individual sample time, which stabilizes model estimation. Because the cortisol diurnal secretory pattern is non-linear, we used a forward selection procedure to fit the log-cortisol curve, arriving at a fourth-degree polynomial. To account for residual dependencies among the log-cortisol values at level 2, we modeled random log-cortisol intercepts for each person.

To test the hypothesis that inaccuracy influences the level or diurnal slope of the modelled cortisol curve, fixed effects were estimated at level 1 with a variable that took the value “1” if the timing of the saliva sample was inaccurate and ‘0’ if it was accurate. The Time by Inaccuracy interaction effect indicates whether the diurnal slope parameter is influenced by

Back to outline

inaccurately timed samples. To test the statistical significance of the regression coefficients, *z*-scores were calculated by dividing the estimated effect by its standard error.
To assess whether inclusion of inaccurately timed samples in the analysis would bias diurnal cortisol profiles, we first used the STATA command LINCOM to calculate 95% confidence intervals for representative time points over the day, based on accurate samples only. We then examined whether the same time points in diurnal cortisol curves based on all samples (inaccurate as well as accurate) fall within the confidence intervals for accurate samples. If so, inclusion of inaccurate samples does not invalidate the results. In addition, we used the same graphical approach to visualize how the cortisol curve estimated from inaccurate samples only deviates from the accurate curve.

RESULTS

Data from three participants were excluded: one participant discarded the electronic monitors and two did not record saliva collection times. On 123 occasions, the monitors were opened without any known reason (there was neither a beep nor a self-reported sample time). On 45 additional occasions subjects opened the monitor and collected a sample in the absence of a valid beep (the watch alarm at 7:30, for example, was sometimes mistaken for a beep). These cases were excluded from the analysis.

Compliance

Samples were missing on 862 occasions when subjects failed to take a saliva sample, including circumstances permitted by the protocol, such as when asleep or when the signal was turned off during a concert. Compliance data are summarized in Figure 1.
Over the 56 subjects, 69 of 1938 of recorded sample times fell outside the time window defined as acceptable (-5, +15). Thus, 96.4% of all samples were adherent with the protocol. Average interval between the programmed beep time and the time recorded for the saliva sample was 3.1 minutes (+/- 3 min SD).

For 152 saliva samples, there was no registered opening of the monitor. Because the timing of the samples could not be verified, we considered these cases as inaccurate. In addition, for 149 samples the time interval between opening of the monitor and self-reported saliva collection fell outside the (-2, +10) interval. The remaining 1568 samples were accurately

Back to outline

timed, representing 83.9% of the adherent cases and 81.0% of all samples taken. The percentage of inaccurate samples averaged 18.8 % per subject (range 0% - 50%). Forty-two subjects had at least one inaccurate sample. On day 1 of the study, 11.8% of the samples were inaccurate; figures for days 2, 3, 4, and 5 were 16%, 13.4%, 22.4%, and 17%, respectively. ANOVA results indicated significant differences in verified compliance rates over the five days of the study ($F(4,55) = 2.8$, $P = .03$). Post-hoc comparisons indicated that non-compliance on day 4 was higher than on day 1; no other comparisons were statistically significant. Non-compliance rates did not vary significantly over the day: figures for beeps 1 through 10 were 18%, 13%, 16%, 18%, 14%, 17%, 14%, 19%, 15%, and 18%, respectively ($F(9,55) = 0.74$, $P = 0.67$).

Impact of inaccurately timed samples on cortisol results

Results of the multilevel regression analysis are summarized in Table 1. Inaccurately timed samples did have small but significant effects on overall cortisol level (estimated effect of inaccuracy) and diurnal slope (inaccuracy by time interaction). These findings do not, however, answer the central and more important question: whether inclusion of inaccurate samples actually distorts estimated cortisol profiles. We therefore contrasted models based on accurate only versus all adherent samples (accurate and inaccurate). Figure 2 shows three log-cortisol curves: (*A*) based on accurate samples only, (*B*) based on inaccurate samples only, and (*C*) based on all adherent samples. Curves falling within the 95% confidence interval (CI) for line A can be considered valid. Consistent with the results shown in Table 1, part of line B falls outside this CI, although a clear diurnal decline is evident. Line C, on the other hand, falls completely within the CI for line A, the most accurate curve. Thus, inclusion of inaccurately timed samples did not significantly distort the estimated cortisol secretory pattern.

DISCUSSION

Extent of protocol adherence and accuracy

Electronic monitoring detected 301 saliva samples with inaccurate or unknown timing that, without monitoring, would have been accepted as valid. This represents a drop from 96.4% self-reported compliance (adherence) to 81% verified compliance (accuracy) for non-missing samples. Verified compliance rates were thus similar to or somewhat better than those previously reported in cortisol studies when subjects were unaware of being monitored (cf.

Back to outline

84% of samples taken within the designated time windows in Kudielka et al., 2003; 80% in fibromyalgia patients and 62% in controls in Broderick et al., 2004). The current study, however, used a narrow time window to define accurate compliance at all times of day: only samples that were actually taken a few minutes before or up to 25 minutes after a programmed beep were considered accurate. If we were to consider samples taken within an hour before or after the signaled time as sufficiently accurate for assessing cortisol levels and diurnal slopes, compliance rates in the current dataset would be at least 89% (all 149 "inaccurate" samples with monitor openings fall within the expanded window).

Evidence suggests that some of the 152 samples (8% of total samples) taken without a registered cap opening may actually have been accurately timed. Several of the 123 unscheduled monitor openings took place in the morning before going to work, and subjects with extra morning monitor openings had more samples taken without a monitor opening later the same day. Most of the self-reported collection times corresponded to actual beep times. Moreover, the similarities in cortisol profiles based on samples defined as inaccurate versus accurate makes it unlikely that subjects who took multiple saliva samples without opening the monitor did so all at once, for example at the end of the day.

Following the ESM protocol, subjects were thus able to collect a large percentage of samples within a very narrow time window, even without knowing that their performance was being monitored. Several features of the sampling protocol may have contributed to good compliance. Firstly, we used a signal-contingent procedure with stratified random sampling times, whereas participants in both previous studies were instructed to take saliva samples at pre-designated times. Fixed time sampling schedules have as drawbacks that subjects are more likely to forget to take a sample and that, having missed a sample, they will find it easier to cheat by "faking" the time of sample collection when planned times are known.

Secondly, the current study had a higher collection load (ten samples a day for five days) than previous studies of compliance (six saliva samples on a single day in the study of Kudielka et al., 2003; five saliva samples each day for seven days in the study of Broderick et al., 2004). Although it seems plausible that compliance might decrease with increased collection load, as suggested by Kudielka et al. (2003), our results indicate otherwise. Accuracy was high and, apart from an unexplained minor dip on day 4, did not decrease systematically over the five-day study. Similarly, Stone et al. (2003) found no significant differences in compliance rates in pain patients signaled 3, 6, or 12 times a day in a stratified random sampling protocol. We speculate that the combination of a high within-day collection load with prompting signals at unpredictable intervals produces a mindset that enhances performance. By focusing attention

Back to outline

on specific moments, the protocol allows the participant freedom from thinking about sampling compliance the rest of the day.

Thirdly, characteristics of participants in our study, in particular the preponderance of twins, may have enhanced compliance rates. A comparison of percentages of accurately reported saliva collection times in twins (N =38) versus non-twin sisters (N = 18) in this sample, however, revealed no significant difference (84% in twins and 82% in sisters, t = 0.58, df=54, p = .56). Another factor that might have increased compliance is past experience as a research subject. Twins recruited via the EFPTS are regularly contacted to participate in studies, whereas non-EFPTS twins are not. Compliance rates were the same (85%), however, in EFPTS and non-EFPTS twins.

Effects of non-compliance on diurnal cortisol profiles

Contrary to previous reports, inclusion of inaccurate samples did not result in blunted diurnal slope measures or other significant differences in estimated cortisol levels. As noted above, compliance rates were higher in the current study, with all samples with verifiable collection times falling within 60 min of the target. In addition, the statistical methods we used insured that the effects of inaccurate samples on cortisol measures depended on their actual frequencies. Kudielka et al. (2003) defined subjects as non-compliant, for the purpose of the cortisol analysis, if they had a least one non-compliant sample and compared compliant versus non-compliant subjects; Broderick et al. (2004) defined days as non-compliant if a subject took at least one non-compliant sample and compared aggregated cortisol profiles on compliant versus noncompliant days in the subset of subjects with both compliant and non-compliant days. The current analysis of cortisol levels was based on compliant versus non-compliant saliva samples. Use of multilevel regression in the statistical analysis (Affleck et al., 1999; Bolger et al., 2003; Schwartz and Stone, 1998) gives a more precise estimate of the effects of non-compliance than methods aggregating data over days or over subjects.

In both previous studies (Broderick et al., 2004; Kudielka et al., 2003) subjects were particularly unsuccessful in complying with early morning samples, unless they knew they were being monitored. The awakening response measure was highly sensitive to inaccurately timed samples, being completely blunted when the “peak” sample was taken more than 40-50 min after the wake-up sample. By defining diurnal slopes in the statistical analysis as the difference between the second (peak) morning measure and the last evening measure, the previous studies appear inadvertently to have confounded the slope measure with the awakening response measure: if the awakening response is blunted as an artifact of samples

Back to outline

that were taken too late, the diurnal change measure will automatically be blunted as well. The current analysis used cortisol data collected 10 times each the day, thus providing a reliable estimate of the diurnal curve. Although multilevel regression results indicated minor differences in level and diurnal slope estimates for inaccurate versus accurate samples, there was no evidence that slopes based on inaccurate samples were blunted.

The current study was not designed to investigate effects of sampling inaccuracy on measures of the cortisol response to awakening. Subjects were free to wake up according to their own schedules and recorded awakening time each day. Average wake-up time was 7:06, with the first saliva sample taken on average at 8:30. If the first ESM beep of the day did happen to occur within an hour after awakening, cortisol values might be higher than in samples at the same time of day but with a longer interval since awakening, as has been reported in studies with a similar design (Peeters et al., 2003). If anything, one would expect that poor compliance in the early morning would be associated with lower cortisol levels, but the multilevel results did not indicate such a pattern. This may be due to the relatively small number of samples taken within an hour of awakening (40 of the 123 first samples of the day).

Self-reported compliance with ESM protocols

The current data were obtained in a study examining emotional and hormonal reactions to daily life stressors. Salivary cortisol was used as an indicator of neuroendocrine response to daily life stress, and at the same time, emotional reactions to daily life stress were measured in ESM self-reports (Peeters et al., 2003; Smyth et al., 1998; van Eck et al., 1996). The current findings showed that subjects adhered to the protocol and were generally accurate in reporting the timing of the cortisol assessment. These results can, with caution, be extrapolated to ESM protocols in general. Because subjects were instructed to collect samples and complete ESM booklets at the same time (and both could be accomplished in less than two minutes), it seems reasonable to assume that accurately timed samples were accompanied by accurately timed ESM reports. Cheating (for example, by taking and labeling the sample at the actual time, entering that time in the booklet, and then waiting to complete the booklet later) would cost considerably more effort than just filling in the booklet at the right time. Moreover, the robust associations between daily experiences and intraindividual mood and symptom fluctuations observed in numerous ESM studies (Myin-Germeys et al., 2001; van Eck et al., 1998) would be improbable if participants consistently failed to complete self-reports on time and were dishonest in reporting actual sampling times.

Back to outline

Although more research is needed, compliance in naturalistic studies with signal-contingent, random time sampling procedures and a high collection load is likely to be satisfactory not only for cortisol assessment, but also for self-report measures. Compliance with fixed time sampling protocols, on the other hand, does appear to be problematic (Stone et al., 2002; Stone et al., 2003). Broderick et al. (2004) reported that self-reported compliance with a fixed time, paper diary protocol with auditory reminding signals was 85%, while verified compliance was only 29 %. Although the above studies attributed the low observed compliance rates to the unmonitored paper diary method, the current findings suggest that their sampling procedures, with fixed times and a low daily sampling load (three diary entries per day), may have been more largely to blame.

Limitations

Given its focus on the extent and effects of not collecting saliva samples at the instructed times, this study does not address the effects of missing data (when samples are not collected at all). Over all subjects, 69% of the theoretical maximum number of samples was collected – a somewhat lower percentage than the rough average of 83% in ESM studies with similar sampling protocols (Peeters et al., 2003; van Eck et al., 1996). It is important to note that ESM protocols typically allow subjects to wake up or go to bed whenever they want and to turn off or remove the signaling device when it would interfere with activities (e.g., in church or when swimming); moreover, there are times when it is impossible to hear the signal (e.g., in traffic). These "legitimate" reasons for missing data are unavoidable in naturalistic studies that encourage participants to carry on their normal daily activities in order to increase the ecological validity of the results. Nevertheless, researchers should remain alert to the possibility that missing data are associated with daily processes under investigation, for example if subjects systematically fail to collect samples when in stressful situations.

The design of the current study did not allow us to assess whether informing subjects of the electronic monitoring would have improved compliance. Based on the two previous studies, we expect that this would indeed be so. In our case, however, the question remains whether the benefits of relatively small improvements in compliance would outweigh the costs of electronic monitoring, as inaccurately timed samples did not significantly affect the cortisol outcome measures. Aside from the expense of the monitors, costs of this methodology include the extra burden on participants of carrying and using the devices. We speculate that this inconvenience might increase the amount of missing data, as the percentage of missing data in the current study was slightly higher than in studies using similar ESM and saliva sampling

Back to outline

procedures but without monitors. New monitoring technologies may help reduce subject burden (see, for example, Stetler et al., 2004).

Recommendations for cortisol sampling in daily life

Based on the available literature, we conclude that electronic monitoring is advantageous for verifying the cortisol response to awakening and for sampling at fixed times of day. In addition, compliance with these sampling protocols is likely to improve if participants are informed of the monitoring. When monitoring of protocol compliance is too costly or inconvenient, the current results suggest that an intensive design (≥ 6 samples per day for at least 2 days), with subjects signaled at unpredictable times, is preferable to the low load, fixed time sampling procedures typically used in studies of diurnal salivary cortisol profiles. Under these sampling conditions, subjects will show better compliance and deviations from the protocol will have less influence on cortisol measures.

CONCLUSION

In this study, compliance with an intensive salivary sampling protocol was high in terms of both adherence and accuracy. In contrast to previous results obtained with sampling at fixed times of day, inclusion of inaccurately timed samples did not distort the cortisol profile. Random time sampling thus appears to have advantages over fixed time sampling for obtaining valid cortisol profiles when researchers do not have devices to monitor compliance. Although further research is needed to test compliance with other sampling protocols and to investigate factors that enhance compliance, these findings indirectly support the validity of momentary self-report data about daily experiences obtained with the same methods.

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Back to outline

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Back to outline

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Back to outline

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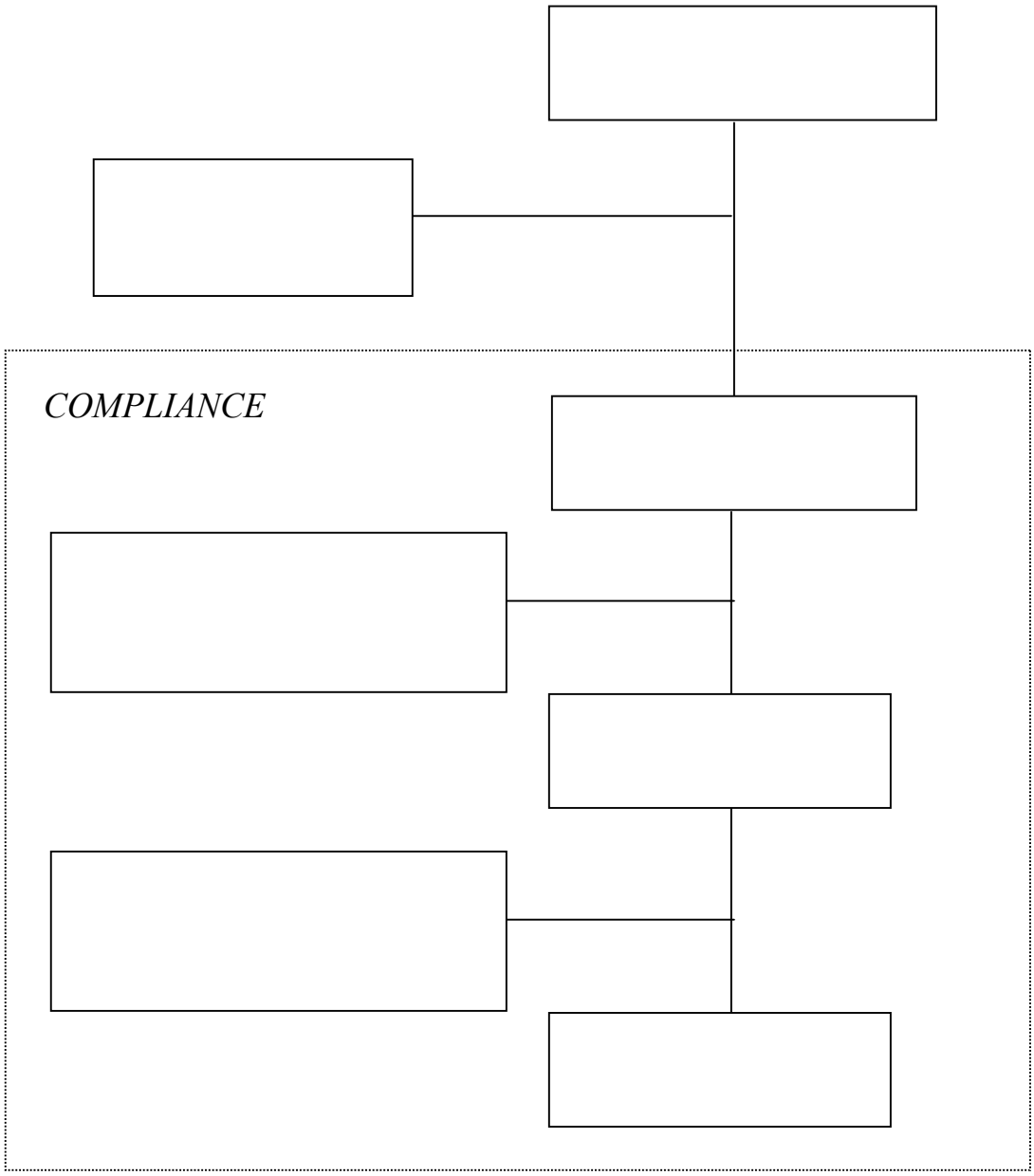
Back to outline

Figure 1 Compliance with the ESM salivary cortisol sampling protocol



Back to outline

Table 1. Multilevel regression estimates (ß) for effects of inaccurately timed saliva samples on the diurnal cortisol curve.

	β (SE)	*Z*	*p*
Overall level (intercept)	1.70 (0.04)	39.54	< .001
Diurnal slope variables			
Time	-0.07 (0.007)	-10.37	< .001
$Time^2$	-0.002 (0.003)	-0.86	0.39
$Time^3$	-0.002 (0.0002)	-9.24	< .001
$Time^4$	0.0001 (0.00006)	2.51	0.012
Inaccurate	-0.089 (0.03)	-2.51	0.012
Inaccurate by Time	-0.02 (0.008)	-2.49	0.013

The dependent measure is log-cortisol. As described in Methods, the time variable was centered around its mean, so that the intercept is an estimate of overall cortisol levels in accurately timed samples. The analysis was based on 1869 salivary cortisol measures nested within 56 subjects.

Back to outline

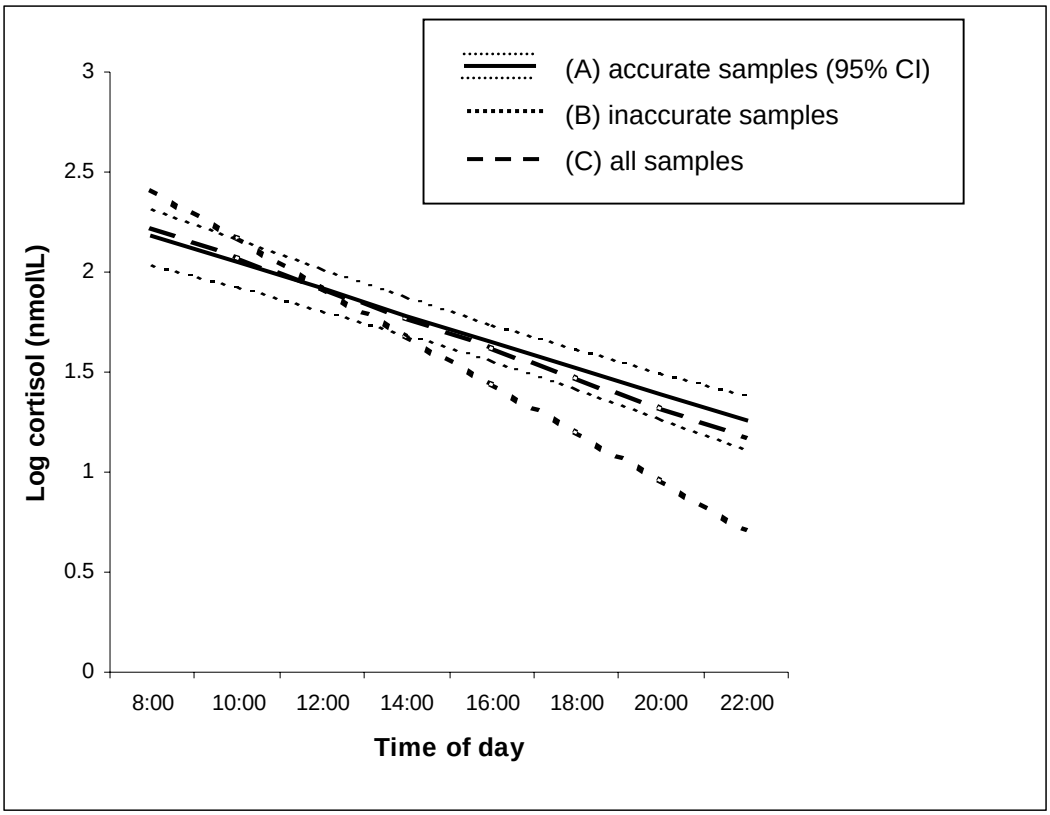


Figure 2. Effects of inaccuracy on modeled diurnal cortisol curves (log transformed values). The solid line A and associated 95% confidence interval were calculated for representative times of day (*x*-axis) from multilevel estimates for cortisol levels in accurately timed samples only (N = 1568). The dotted line B shows modeled values for inaccurately timed samples only (*N* = 301); the dashed line C shows values for all adherent samples, accurate and inaccurate (*N* = 1869).

Back to outline

Chapter 7:

A momentary assessment study of the relationship between affective and neuroendocrine stress responses in daily life

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Biological Psychology, submitted

Back to outline

Abstract

It has been suggested that the accumulation of stress-induced cortisol responses, which is thought to increase the risk for mental health outcomes such as depression, is mediated by the tendency to experience negative emotions in the face of stress. Momentary assessment methodology was used to measure stress as ongoing activity related stress and examine the relationships between stress on mood and HPA-activation.

The Experience Sampling Method **(**ESM), a validated structured diary technique to assess stressors, mood and salivary cortisol at 10 random times daily for 5 consecutive days, was used in a general population sample of 556 women. Multilevel analysis was applied to investigate associations between ongoing stress, mood (negative affect, positive affect, agitation) and cortisol levels.

Minor daily life stress was associated with decreased positive affect, increased negative affect and increased agitation, but only negative affect was independently associated with increased cortisol levels. In addition, negative affect mediated part of the effect of stress on cortisol.

The tendency to experience negative affective states in the flow of daily life likely explains part of the pathway whereby cumulative stress-induced cortisol responses shape the risk for mental health disorders.

Back to outline

Introduction

Numerous studies have shown that stressful life events are associated with the onset, course and relapse of major depression (Brostedt and Pedersen, 2003; Brown, 1981; Kendler et al., 1999; Paykel, 1978). There is evidence that the association between stress and affective dysregulation is in part mediated by stress-induced increases in hypothalamic-pituitary-adrenal axis (HPA) activation (Van Praag, 2004; Van Praag et al., 2004). Increased biological stress responsiveness prepares the individual to cope with the stressor (De Kloet, 2004; Young et al., 2004), but hyperactivation of this system is thought to be involved in the pathogenesis of major depression (Muller et al., 2002; Parker et al., 2003; Strohle and Holsboer, 2003; Tafet and Bernardini, 2003).

In an attempt to further elucidate the relationship between stress, affective appraisals and neuroendocrine responses, more recent studies, rather than focusing on the impact of relatively rare but major stressful life events (e.g. job loss, death of a loved one or major financial problems), have examined the effects of minor stressful events or daily hassles. Although the impact of daily hassles may be smaller, they occur much more frequently and can, therefore, have a substantial cumulative effect on mental health. Momentary assessment methods have been developed to study these minor daily hassles in relation to daily life fluctuation of subjective mood states and behaviour (Csikszentmihalyi and Larson, 1987; Delespaul, 1995; deVries, 1992; Larson and Csikszentmihalyi, 1983), allowing recently validated empirical work (Jacobs et al., in press) relating point assessments of subjective mood states with point assessments of adrenocorticol responses in daily life using salivary cortisol sampling techniques (Kirschbaum and Hellhammer, 1994b; Nicolson, 1992; Ockenfels et al., 1995; van Eck et al., 1996a). Research employing such momentary assessment methodologies has shown that minor stressful daily events are associated with changes in mood in that positive affect decreases, whereas negative affect and agitation increase (Delespaul, 1995; Gable et al., 2000; Marco et al., 1999; Marco and Suls, 1993; Myin-Germeys et al., 2003; Smyth et al., 1998; van Eck et al., 1998; Zautra et al., 2000). In addition, minor daily stress has been shown to activate the HPA-axis as evidenced by stress-related increases in cortisol levels (Ockenfels et al., 1995; Peeters et al., 2003a; van Eck et al., 1996a). The association between stress in daily life and cortisol, however, appears to be indirect and mediated by negative affect and agitation (Peeters et al., 2003a; Smyth et al., 1998; van Eck et al., 1996a), providing support for the view that the tendency to experience negative emotions, a trait that is strongly linked with depression genetically (Kendler et al.,

Back to outline

1993) and longitudinally (Van Os and Jones, 1999), is central in the pathway linking stress, cortisol and mental health outcomes (Van Praag, 2004; Van Praag et al., 2004).

All the above studies on daily life stress, mood and salivary cortisol response depended on the exclusive requirement that, according to the ESM protocol, subjects make two conscious appraisals. First, at each " beep" (the random signal involved in experience sampling, typically 10 times per day for 5-7 days), the subject was asked to identify two experiences that occurred over the past 5 to 90 minutes, and assign it to pre-existing categories of "positive" or "negative" events. Second, for both the negative and the positive event, the subject was required to make a conscious appraisal of its degree of "stressfulness". It has been argued that the requirement to consciously and compulsorily allocate and appraise experiences to pre-existing categories of events and levels of stressfulness may introduce error (Stone et al., 1998). For example, individuals may not remember an event, or they may, because of a current activity or mood, not label a past experience as an "event". Similarly, subjects may label a past event as stressful or negative under the influence of an ongoing stressor. An alternative, therefore, is to define minor daily life stress as *ongoing* activity related stress without the requirement to label past experiences as either "positive" or "negative" and without the requirement to consciously appraise the level of "stressfulness", thus avoiding the problems associated with retrospective recall and interpretation.

The aim of the current study was to examine the impact of ongoing activity-related stress on mood and HPA-axis activation, in a large general population sample of women, using a momentary assessment design. It was hypothesized that (1) ongoing activity-related stress would be associated with changes in mood (decrease in positive affect, increase in negative affect and agitation) and cortisol and (2) negative affect and agitation (and not positive affect) would be associated with elevated HPA-activation and (3) both negative affect and agitation would mediate the relation between ongoing activity related stress and cortisol secretion.

Back to outline

Method

Sample

The sample consisted of 549 members of Belgian female twin pairs and 47 of their sisters and has been described before in detail in a previous paper validating the ESM and salivary cortisol sampling procedures used in this study {for details, see Jacobs et al, in press life sciences}. Mean age of the sample was 27 years (SD 8 years; range 18-61), 36.9 % completed secondary school and 62.5% had a college degree. The majority was currently employed (64.5% employed, 30.4% student, 2.5% homemaker, 2.6% unemployed).

Experience Sampling Method

A trained research assistant visited each participant at home to explain the study procedures in detail. Subjects wore a digital wristwatch programmed to emit a signal ("beep") at an unpredictable moment in each of ten 90-minute time blocks between 7:30 and 22:30, on five consecutive days. After each beep, subjects completed a short self-report questionnaire in a pocket-sized booklet, with open questions and rating scales concerning current thoughts, mood, activity as well as physical and social context. At the same time, they collected a saliva sample with a cotton swab (Salivette; Sarstedt, Etten-Leur, the Netherlands). After saliva collection, subjects were instructed to store the swab in the salivette tube and to record the exact time of collection on the label, on which subject code and date had been pre-printed. Samples were stored in subjects' home freezers until transport to the lab, where uncentrifuged samples were kept at –20° C until analysis.

Measures

Both the mood measures and the stress measure were derived from the experience sampling reports as described below and described in previous papers with different samples (Myin-Germeys et al., 2003; Myin-Germeys et al., 2001).

Stress was conceptualised as the subjectively appraised "stressfulness" of the ongoing activity. To assess "stressfulness" without priming individuals, this was assessed indirectly with 2 items rated on 7-point Likert scales (from 1 = 'not at all' to 7 = 'very'). The mean of the items '*I would rather be doing something else*' and '*this activity requires effort*' formed the STRESS scale (Cronbach's alpha = 0.57).

Mood states were assessed with 13 mood adjectives rated on 7-point Likert scales (from 1= 'not at all' to 7 = 'very'). The mean of the adjectives '*cheerful*', '*satisfied*', '*energetic*' and '*enthusiastic*' formed the Positive Affect (PA) scale (Cronbach's alpha = 0.86). The Negative

Back to outline

Affect (NA) scale consisted of the mood items '*insecure*', '*lonely*', '*anxious*', '*blue*', '*guilty*' and '*suspicious*' (Cronbach's alpha = 0.76) and the mean of the adjectives *'calm', 'relaxed' and 'harried'* constituted the Agitation (AG) scale (Cronbach's alpha = 0.72).

Salivary cortisol (CORT) is a valid, reliable and non-invasive measure of the free, unbound cortisol in blood, which is considered to be the biologically active hormone. Cortisol, an end product of the hypothalamic-pituitary-adrenal axis, is known to reflect the individual's response to psychological and physiological stress (Kirschbaum and Hellhammer, 1989, 1994a).

Salivary free cortisol levels were determined in duplicate, using a time-resolved immunoassay with fluorescence detection (Dressendorfer et al., 1992). The lower detection limit of this assay was 0.2 nmol/L; interassay and intra-assay coefficients of variation were less than 10%. The raw cortisol values were log transformed to reduce skewness of their distribution.

Analysis

ESM data have a hierarchical structure. In this study, multiple observations (level 1) are nested within days (level 2), which are clustered within subjects (level 3), who are part of twin pairs (level 4). Multilevel analysis takes the variability associated with each level of nesting into account (Snijders and Bosker, 1999). The βs are the regression outcomes of the predictors in the multilevel model and can be interpreted identically to the estimates in standard (single level) regression analyses. To investigate the effect of STRESS on mood (PA, NA, AG), multilevel linear regression analyses, using MlwiN (Rasbash et al., 2000; Rasbash et al., 2001), were performed with STRESS as the independent variable and NA, PA and AG respectively as dependent variables. Next, a multilevel linear regression analysis with STRESS as independent variable and CORT as dependent variable was carried out to examine the effect of STRESS on CORT. To investigate the extent to which mood mediated any association between STRESS and CORT, a multilevel analysis with CORT as dependent variable and STRESS, NA, PA and AG as independent variables was performed.

All variables were standardised (e.g. standardised NA = NA/Standard Deviation (SD) of NA in the whole sample). Thus, the effect of the independent variable (STRESS) was expressed in units SD of the dependent variable (NA, PA, AG and CORT). According to Cohen (Cohen, 1988), 0.8 SD can be considered as a large effect size, and 0.2 SD as a small effect size.

The multilevel analyses with CORT as dependent variable were corrected for the following *a priori* confounders: diurnal pattern of cortisol (represented by a fourth-degree polynomial, (Peeters et al., 2004)), recent consumption of food or alcohol, tobacco use and use of steroid

Back to outline

medications, including oral contraceptives (other possible confounders such as consumption of coffee, level of physical activity, medication intake and depressive symptoms, as measured by the 90-item Symptom Check List, (Derogatis et al., 1976) were not found to have significant associations).

Results

Sample

Of the 596 participants, 3 were excluded because of pregancy and 27 were excluded because of use of antidepressants or other psychoactive drugs at the time of the study. In addition, 6 participants were excluded because of an insufficient number of valid observations (*a priori* defined as < 17 of the maximum of 50 beeps within the time window [-5, +15] minutes of the beep) (Delespaul, 1995), and 4 subjects were excluded because of extreme cortisol values (*a priori* defined as > 20% of the valid saliva samples having cortisol > than 44 nmol\L). Out of the maximum of 28300 observations (556 subjects x 10 beeps x 5 days), 16536 observations (58.4%) had complete data (measures of STRESS, PA, NA, AG and CORT as well on consumption of food and alcohol, tobacco use and medication). These observations formed the dataset for all analyses.

Aggregated over participants' means, mean raw PA was 3.55 (SD: 0.69; range: 0.86-5.48), mean raw NA was 0.82 (SD: 0.25; range: 0.65-2.57), mean raw AG was 2.02 (SD: 0.53: range 0.77-3.76) and mean raw STRESS was 1.78 (SD: 0.5; range 0.8-0.3.58).

Affective sensitivity to minor daily life stress

The multilevel analyses showed a significant association between STRESS and PA (ß = –0.19, $p < 0.001$), STRESS and NA (ß = 0.15, $p < 0.001$) and STRESS and AG (ß = 0.24, $p < 0.001$), indicating that increases in STRESS were associated with significant decreases in PA and significant increases in both NA and AG.

Neuroendocrine sensitivity to minor daily life stress

The multilevel analysis showed a significant association between STRESS and CORT (ß = 0.01, $p = 0.02$). No significant association was found between PA and CORT (ß = –0.0004, $p = 0.92$), but both NA and AG were significantly associated with CORT (respectively ß = 0.03, $p < 0.001$; ß = 0.02, $p = 0.007$). However, the significant association between AG and CORT disappeared when NA was controlled for (ß= 0.009, $p = 0.15$), whereas the association with NA remained unchanged (ß= 0.03, $p < 0.001$).

Back to outline

Mood mediation

To investigate whether mood mediated the association between STRESS and CORT, a multilevel analysis was carried out with CORT as dependent variable and STRESS and NA as independent variables. Given the absence of an independent association with CORT, PA and AG were not included in these analyses. The multilevel results showed an unchanged effect of NA, both in terms of strength of association and statistical significance ($ß = 0.03$, $p < 0.001$), whereas the independent effect of STRESS was reduced by about 20% and was no longer significant ($ß = 0.008$, $p = 0.13$).

Discussion

Minor daily stress was associated with changes in mood (PA, NA and AG), but only NA was independently associated with increased cortisol levels. In addition, changes in NA accounted at least in part for the association between STRESS and CORT, as STRESS was no longer a significant predictor of CORT after controlling for NA.

Affective sensitivity to minor daily life stress

The first hypothesis was confirmed, in that daily life stress caused changes in mood (decrease in PA, increase in NA and AG), in line with previous research. Myin-Germeys and colleagues, using a similar measure of stress, reported that an increase in stress was associated with increased NA and decreased PA (Myin-Germeys et al., 2003; Myin-Germeys et al., 2001). This pattern was also found in several other studies, using different measures of stress (Gable et al., 2000; Marco et al., 1999; Peeters et al., submitted; Peeters et al., 2003b; Smyth et al., 1998; van Eck et al., 1998; Zautra et al., 2000). In addition, Van Eck and colleagues (van Eck et al., 1998) showed that negative events were followed by increases in AG (as well as NA), which was confirmed in our study.

In contrast to previous studies, this study focused on ongoing, activity related stress.

It is remarkable that even these minor stressors, occurring very frequently in everyday life, have a significant effect on mood, suggesting that the subjective affective appraisal of a stressful event, rather than its occurrence as such, is an important determinant of its impact on the individual (Lazarus and Folkman, 1984).

Back to outline

Neuroendocrine sensitivity to minor daily life stress

The results also supported the second hypothesis. PA showed no significant association with cortisol, but NA and AG were associated with elevated cortisol levels. However, when NA and AG both were included in the regression analyses, only NA remained as predictor, with a small, but statistically significant effect. Cortisol secretion was influenced by NA only. NA and PA are considered to represent the affective elements of an evolutionarily adaptive system that mediates withdrawal and goal-directed behaviors (Watson, 2000; Watson et al., 1999). The basic function of PA is to direct organisms "towards situations and experiences that potentially may yield pleasure and reward" (Watson, 2000), whereas NA levels are more specifically reactive to threatening and aversive stimuli (Watson et al., 1999). Stress can be considered such an aversive stimulus, resulting in elevated NA levels pushing organisms to react in order to eliminate its (potentially) damaging effects. In order to achieve this goal, behavioural action is required and biological stress responses such as increased cortisol secretion are turned on (De Kloet, 2004; Young et al., 2004).

The tendency to experience stress-induced negative emotional states and mental health

Minor daily life stress was associated with mood changes and HPA-axis activation. Together with other studies (Jamner et al., 1991; Myin-Germeys et al., 2003; Peeters et al., 2003b; Stone et al., 1996; van Eck et al., 1996a; van Eck et al., 1998), these results indicate that stressors in daily life are capable of influencing neuroendocrine, and, by implication, cardiovascular and immunological response systems. Thus implicating daily life stress in the onset and course of many common illnesses in particular depression (Myin-Germeys et al., 2003; Peeters et al., 2003a; Sher, 2004; van Eck et al., 1998). In addition, emotional responses to stress mediated the neuroendocrine response. When negative affect was taken into account, the effect of stress on cortisol was reduced by about 20% and was not significant. The effect of stress on cortisol therefore appears to be mediated to a large extent by associated increases in NA, as reported in earlier work (Peeters et al., 2003a; van Eck et al., 1996a), showing similar reductions when controlling for NA. The current results therefore confirm that the tendency to experience negative emotions in the face of stress is of central importance in the link between minor daily life stress and cortisol-related health outcomes, and may explain consistent findings linking the tendency to experience negative emotions, or neuroticism, early in life and later mental health outcomes such as not only depression but also schizophrenia (Goodwin et al., 2003; Krabbendam et al., 2002; Rodgers, 1990; Van Os and Jones, 1999, 2001). A large degree of the genetic liability for depression is shared with

Back to outline

negative affectivity (Kendler et al., 1993). Genetic analysis of the data pertaining to this sample showed that 55% to 68% of the inter-individual variation in affective stress reactivity was explained by genetic factors and 32% to 45% was explained by individual-specific environmental factors (Jacobs et al., under review). Identifying and modifying these factors, if possible, will therefore further increase our understanding of affective stress reactivity and yield possible avenues to reduce the risk of mental health disorders.

Methodological issues

This study examined affective and neuroendocrine sensitivity to minor daily life stress in a large female sample. Previous research reported gender differences in intensity of affect (Fujita, Diener and Sandvik, 1991) as well as in cortisol response to stress (Kirschbaum et al., 1995a; Kirschbaum et al., 1995b; Kirschbaum et al., 1992; Smyth et al., 1998). Women reported higher NA and more intense PA then men, but men showed larger cortisol response to stress than women. Therefore, a replication of this study in males is required. Even so, Van Eck and colleagues conducted analyses a male sample and although daily stress was defined in a different manner, the results of their analyses are in line with the findings of the current study (Peeters et al., 2003a; van Eck et al., 1996a; van Eck et al., 1998; van Eck et al., 1996b). The sample used in this study was representative of the general population, limiting generalisability to clinical populations, as people suffering from psychiatric disorders such as major depression, express different patterns of stress responses (Myin-Germeys et al., 2003; Peeters et al., 2003a; Peeters et al., 2003b). Although a causal pathway between stress, mood and cortisol is assumed in the analyses, causality cannot be firmly proved as data about stress, mood and cortisol were collected at the same point in time. However, Peeters and colleagues (Peeters et al., 2003b) showed that prior events, controlled for the effect of current event, were significantly associated with changes in mood. In addition, studies examining short-term, low-dose prednisone administration (a corticosteroid presumed to have similar effects as endogenous cortisol) showed no effect on self-rated mood (Brown and Suppes, 1998; Warot et al., 1995; Wolkowitz et al., 1990). Long-term, high dose prednisone on the other hand is associated with mood changes, in particular with increased self-reported negative emotions (Bolanos et al., 2004; Schmidt et al., 1999). This study focused on minor daily stressors of which the effects on the HPA-axis are more comparable to the effect of short-term, low-dose prednisone administration rather than long-term, high-dose administration. The results therefore suggest that minor daily stress influences mood rather than vice versa.

Back to outline

Acknowledgements

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Back to outline

Figure 1 : *Affective and neuroendocrine responses associated with minor daily activity-related stress*

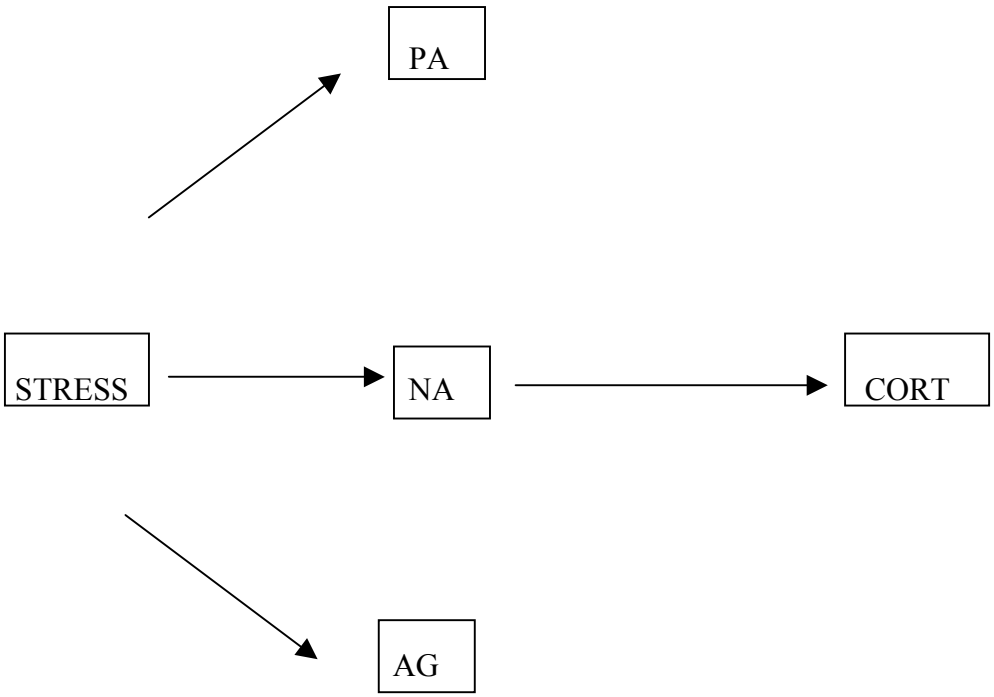

Figure Legends

PA: positive affect; NA: Negative affect; AG: Agitation; CORT: Cortisol

The values shown are statistically significant (standardized) regression coefficients, as estimated in the multilevel models.

Back to outline

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Back to outline

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Back to outline

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Back to outline

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Back to outline

Chapter 8:

Genes making one feel blue in the flow of daily life: A momentary assessment study of gene-stress interaction.

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Back to outline

ABSTRACT

Individual differences in stress-reactivity constitute a crucially important mechanism of risk for depression. As stress is better conceptualized as the continuous occurrence of minor daily hassles, this study focused on emotional reactivity to stress in the flow of daily life and examined to what degree individual differences in emotional reactivity could be explained by genetic and/or environmental factors.

275 female twin pairs (170 monozygotic and 105 dizygotic) participated in this Experience Sampling study (ESM). ESM is a validated structured diary technique assessing stressors and mood in daily life. Individual emotional stress-reactivity was conceptualised as changes in negative affect in relation to minor daily life stressors. Structural equation modeling was used to fit univariate models. The best fitting model was chosen, based on likelihood and parsimony.

Genetic factors (explaining 55% to 68% of individual differences) and individual-specific environmental factors (explaining 32% to 45% of individual differences) influenced daily life stress-reactivity. The best fitting model also incorporated negative sibling interaction.

The demonstration of a genetic influence on the dynamic relationship between minor stress and affective response in the flow of daily life sheds light on the gene-environment interactions that drive the risk to develop stress related disorders such as depression. Differences in stress-reactivity between children in the same family may result in part from compensatory sibling interactions.

Back to outline

INTRODUCTION

Stress is inevitably imbedded in the flow of daily life and has a strong impact on general health. Prolonged stress is associated with decreased well-being, increased sick leave rate and the development of stress-related disorders such as burn-out and depression (1-4). Stress is therefore expected to be one of the major causes of dysfunction in the coming decades (5). People, however, greatly differ in the way they respond to stress, some being more susceptible to its effects than others (6-11). In the current paper, the origin of these individual differences in sensitivity for stress was investigated.

Stress research originally focused on the occurrence and frequency of relatively rare stressors such as major life events (e.g. job loss, death of a loved one or major financial problems) (9, 12, 13). More recently, however, it has been suggested that stress as an exposure is better conceptualized as the continuous occurrence of minor daily hassles and their emotional impact. Momentary assessment methods have been developed to assess the dynamic relationship between these minor daily hassles and momentary fluctuation in mood states in the flow of daily life (14, 15). Research employing such momentary assessment methodologies has provided evidence that small stressors occurring in the flow of daily life are associated with psychological and somatic dysfunction (16-19) and that the individual's emotional appraisal of a stressful event, rather than its occurrence as such, is crucial in determining its subsequent impact (20). For example, affective stress-responses, in particular momentary changes in negative affect, were found to occur in parallel with the biological, cortisol-mediated, stress response indicating that variability in emotional stress-reactivity is a crucial marker of individual differences in sensitivity to stress (21, 22). These findings are in line with the proposed importance of momentary variations in negative affect as a genetically influenced trait conferring evolutionary advantage in the face of the ever changing demands and threats facing the individual in the flow of daily life (23)

The aims of the current study were to quantify individual differences in emotional reactivity to stress in the flow of daily life and to determine its underlying sources of variation. As individual differences can be caused by either genetic factors or by environmental factors, twin studies allowing for the separation of these effects are an appropriate means to this end, in particular as there have been no previous studies of the relative contributions of genes and environment to individual differences in emotional stress-reactivity in the flow of daily life. In a sample of 272 female twin pairs, it was examined to what degree emotional reactivity to daily stress can be explained by genetic factors and / or by environmental factors.

Back to outline

METHOD

Sample

The study sample consisted of 275 female twin pairs between 18 and 46 years of age from Flanders (Belgium). Two-hundred and eighteen pairs came from the East Flanders Prospective Twin Survey. This population-based survey has prospectively recorded all multiple births in the province of East Flanders since 1964 (24, 25). Perinatal data were collected at birth, and placental examination was performed within 48 hours after delivery. Zygosity was determined through sequential analysis based on sex, fetal membranes, blood groups and DNA fingerprints. Fifty-seven pairs were recruited using registers from Flemish municipalities. Determination of zygosity in these twins was based on self and mother's report of standard questions about physical similarity and the degree to which the twins are confused (26-28) and, if necessary, on examination of DNA fingerprints. The project was approved by the Local Ethics Committee and all participants gave written informed consent.

Momentary assessments method

The Experience Sampling Method (ESM) is a structured diary technique to assess subjects in their daily living environment. It is a valid and reliable way to study immediate effects of stressors on mood, reducing biases in recall (14, 15, 29). Subjects received a digital wristwatch and a set of ESM self-assessment forms collated in a booklet for each day. The wristwatch was programmed to emit a signal ("beep") at an unpredictable moment in each of ten 90-minute time blocks between 7:30 and 22:30, on five consecutive days. After every beep, subjects were asked to stop their activity and to fill out the ESM self-assessment forms previously handed to them, collecting reports of thoughts, current context (activity, persons present, location), appraisals of the current situation and mood. All self-assessments were rated on 7-point Likert scales. Trained research assistants with experience in momentary assessment techniques explained the ESM procedure to the participants during an initial briefing session and a practice form was completed to confirm that subjects were able to understand the 7-point Likert scale. Subjects could call a telephone number in case they had questions or problems during the ESM sampling period. Subjects were instructed to complete their reports immediately after the beep, thus minimizing memory distortions, and to record the time at which they completed the form. In order to know whether the subjects had completed the form within 15 min of the beep, the time at which subjects indicated they completed the report, was compared to the actual time of the beep. A momentary assessment

Back to outline

validation study in this sample showed that random sampling procedures with high sampling loads yielded compliance rates in excess of 90% (30). All reports not filled in within 15 minutes after the beep were excluded from the analysis. Previous work (15) has shown that reports completed after this interval are less reliable and consequently less valid. Subjects with less than 17 valid reports were excluded from the analysis.

Momentary assessment measures

Emotional stress reactivity was conceptualised dynamically as mood reactivity to minor disturbances in daily life (see (19, 31). Both the mood measures and the stress measure were derived from the Experience Sampling reports as described below.

Conform previous work (19, 31), minor daily life stress (STRESS) was conceptualised as the subjective appraised stressfulness of the activity that was going on at the time of the ESM report. It was assessed with two items ('I would rather do something else' and 'this activity requires effort') rated on 7-point Likert scales (from 1= 'not at all' to 7 = 'very'). The mean of the two items forms the STRESS scale (Cronbach's alpha = 0.57 over the subject mean).

Negative Affect (NA) was assessed with 6 mood adjectives (I feel 'insecure', 'lonely', 'anxious', 'low', 'guilty' and 'suspicious') rated on 7-point Likert scales (from 1= 'not at all' to 7='very'). The mean of the 6 items forms the NA scale (Cronbach's alpha = 0.76 over the subject mean).

In order to obtain a measure of stress-reactivity for each subject, regression analyses with NA as the dependent variable and STRESS as the independent variable was conducted for each subject separately (over the 50 reports of each subject). The regression coefficient of this equation represented the individual change in NA associated with the subjective appraisal of minor daily STRESS and was used in the analyses as the measure of individual stress-reactivity.

Analyses

Correlation analysis

Comparison between MZ and DZ intrapair correlations of the stress-reactivity measure gives a first impression about the role of genetic and environmental factors. If the MZ intrapair correlation is about twice as high as the DZ intrapair correlation, additive genetic factors (A; the sum of the average effects of the individual alleles at all loci affecting the phenotype) and individual-specific factors (E; environmental influences that are not shared between family members) are likely to play a role in determining individual differences in the trait. If, on the

Back to outline

other hand, the MZ intrapair correlation is much higher than twice the DZ intrapair correlation, additive genetic factors (A), dominant genetic factors (D; effect of interacting genes present at the same chromosomal locus) and individual-specific factors (E) are likely to explain the variation within the variable. However, this pattern can also be indicative of sibling interaction (i.e. the behaviour in one twin directly affects behaviour in the co-twin) if difference in phenotypic variance between MZ and DZ twins is observed (32, 33). Negative sibling interaction is suggested if the MZ variance is significantly lower than the DZ variance and, conversely, if the MZ variance is significantly higher than the DZ variance positive sibling interaction may be present. In the case of a 'true' dominance genetic effect, MZ and DZ variances are expected to be of equal magnitude.

Structural equation model fitting

Based on the results of the correlation analysis, univariate structural equation models (SEM) were applied using the program Mx (34). The goal of univariate genetic model fitting is to decompose the phenotypic variance into the possible sources A, D, E and sibling interaction effect (SI), if applicable (see Figure 1).

Model parameters are estimated by minimising a goodness-of-fit statistic (χ^2) between observed and model-predicted covariances. Raw data analysis was used to handle missing data problems. When analysing models with raw data, minus twice the log-likelihood (-2*LL) of the data for each observation is calculated. This implies that there is no overall measure of fit, but rather relative measures of fit, since differences in fit function between sub-models are distributed as χ^2. The goodness-of-fit of e.g. the full ADE model is measured relative to a perfect fitting (saturated) model. In a saturated model, the maximum number of parameters is estimated to describe the correlational structure between variables. For example, for the covariance matrix in one group (MZ or DZ) that would be 5 parameters: 2 variances (of twin1 and twin2); 1 cross-twin covariance and 2 means.

A non-significant χ^2 value suggests that the model is consistent with the data, whereas a significant χ^2 value ($p<0.05$) suggests that the model poorly fits the data and can be rejected. Goodness-of-fit of alternative, nested models were evaluated by changes in χ^2. For example, the fit of a reduced model (e.g. AE) will be better (i.e. the dropped parameter D will be non-significant) if the difference in χ^2 for 1 degree-of-freedom does not exceed the critical value (at the .05 level) of 3.84. Information about the precision of parameter estimates (and their explained variance) was obtained by likelihood-based Confidence Intervals (CIs) rather than standard errors (35).

Back to outline

Allowing for sibling interaction effects in the model has as consequence that the total variance of a phenotype becomes dependent on the genetic relationship of the subjects, resulting in different standardized estimates of A, D and E for MZ and DZ twins (32, 33).

RESULTS

Sample

275 female twin pairs participated: 170 monozygotic and 105 dizygotic. Fourteen subjects were excluded because they had fewer than 17 valid self-reports. Mean age of the twins was 27 years (SD: 7.4 years, range 18-46 years); 62.5% had a college or university degree, 35.1% completed secondary education and 2.4% had a primary education. The majority was currently employed (63.0% employed, 32.2% student, 2.4% unemployed, 2% housewife and 0.4% sick leave).

Stress-reactivity measure

The stress-reactivity coefficient was not normally distributed [$p_{skweness} < 0.05$; $p_{kurtosis} < 0.05$; median = 0.02 (95% C.I. 0.015-0.03)], and log-transformed [ln (stress-reactivity coefficient x 100 +15)] to reduce skewness ($p_{skweness} = 0.13$; $p_{kurtosis} < 0.05$). The mean of the transformed stress-reactivity coefficient was 2.91 (std = 0.38).

Analyses

Correlation analyses

The Spearman MZ intra-pair correlation equaled 0.17 (p=0.03), whereas the Spearman DZ intrapair correlation was found to be –0.1 (p=0.27). This pattern suggests a dominant genetic influence on the phenotype. However, the significantly lower MZ variance as compared to the DZ variance (std MZ=0.34 < std DZ=0.44; $p < 0.05$), suggested the effect of negative sibling interaction.

Structural equation model fitting

The first model tested was the fully saturated phenotypic model to which all genetic models were compared in order to derive a chi-square fit-index and p-value. The second model was a full genetic ADE-SI model. The estimates of both A and D in this model were non-significant, i.e. 95% CI include 0, but they could not be dropped simultaneously [$\Delta\chi^2(2)=20.5$, $p<.001$, E-SI model]. The SI factor was estimated at –0.32 and was significant (95% CI –0.42;-0.17).

Back to outline

Given the lack of power to identify non-additive effects (in combination with additive effects) (36), and in combination with sibling interaction effects (33), we regard the estimates of the more parsimonious AE-SI model here, although this model, strictly speaking, cannot be selected as the best-fitting model. This will at least allow us to discuss possible trends in our data. It should be noted that the fit of all genetic models were significantly worse compared to the fit of the fully saturated model. This is probably due to slight violations of the distributional assumptions.

Modeling a sibling interaction effect leads to different standardized parameter estimates for MZ and DZ twins. The standardized effect of the additive genetic factor was estimated at 0.55 for MZ twins (95% CI: 0.44-0.63) and 0.68 for DZ twins (95% CI: 0.52-0.77).

DISCUSSION

This study focused on the origin of individual differences in stress-reactivity in daily life, which was defined as stress-induced momentary increases in negative affect. Univariate structural equation modeling showed that both genetic and individual-specific environmental factors play a role in daily life stress-reactivity. The results also provided evidence for negative sibling interaction, indicating that stress-reactivity in one twin resulted in reduced stress-reactivity in the co-twin.

Gene-stress interaction

As our stress reactivity measure included the interaction between stressor and emotional response, the demonstration of a genetic influence on this dynamic relationship is indicative of gene-environment interaction: genes contribute to individual differences in emotional sensitivity to stress (37). Genes therefore co-determine momentary emotional reactivity to stresses in the immediate environment in the flow of daily life. Up to 55% – 68% of the total variance was explained by genetic factors, indicating that genes have a substantial impact on differences between people with respect to sensitivity for the negative effect of minor daily events on mood. Increased stress-reactivity in daily life is associated with increased risk for depression, bipolar disorder and non-affective psychotic disorder (31, 38). The current study, thus, suggests that the genes that put subjects at risk to develop these disorders do so in part by increasing stress-sensitivity in the flow of daily life.

As genetic factors were found to play an important role, it is important to identify the genes that influence daily life stress-reactivity. A possible candidate is a functional polymorphism

Back to outline

on the serotonin transporter gene (SERT). It has been demonstrated that SERT sites in the human brain are associated with emotional functions (39). In addition, there is evidence that SERT might be involved in stress-related disorders such as psychotic illness (40) and the tendency to develop depression after exposure to major life events (11). The measures used in the current study are quite different in that not major life events, but small stressors in the flow of daily life were assessed as the exposure and not clinical depression but momentary changes in negative affect as the outcome. A next urgent step therefore is to examine the role of SERT in moment-to-moment changes in negative affect in the face of minor stressors in daily life. A positive association between a functional SERT polymorphism and daily life stress sensitivity could help to further unravel the genetics of selected traits that enhance environmental adaptation on the one hand but could also increase the risk of disorders such as depression on the other. A functional polymorphism on the MAO-A gene has also been reported to moderate children's sensitivity to maltreatment (41), which makes MAO-A another suitable candidate gene. The MAO-A gene encodes the MAO-A enzyme which catabolizes several neurotransmitters among which serotonin. MAO-A and SERT are, therefore, also possible candidates to examine epistatis or gene-gene interaction. However, Caspi et al. found that the moderation of major life events on depression was specific to the SERT-polymorphism and independent of the individual's MAO-A gene status (11). This, however, does not preclude interaction using momentary assessment measures of stress and mood-reactivity which are quite different from the measures of major life events and clinical depression used in the work reported by Caspi et al. (11).

Although ours and previous findings need to be replicated, they all show substantial evidence for genotype-environment interaction and they even suggest possible gene-gene interaction. This has implications for traditional molecular genetic studies. Instead of searching for genes directly associated with the phenotype, molecular genetic studies may profit from incorporation of environmental factors in the research design and identify genes which are indirectly (via environmental factors) associated with the phenotype (42).

The other 32 to% – 45% of the total variance of stress-reactivity was explained by individual-specific environmental factors (and measurement error). Social support may be an example of such an individual-specific environmental factor. It is well-known that social support influences the way in which individuals react to stressors (43, 44), thus in fact representing an environment-environment interaction.

Back to outline

Sibling interaction

The best fitting model also included negative sibling interaction, indicating that increased stress-reactivity in one twin resulted in reduced stress-sensitivity in the other. It is unclear whether this should be understood as a pattern of immediate interaction between the twins (which is plausible as twins usually have frequent contacts) or whether this reflects interaction patterns during childhood resulting in different personality styles in adulthood. Incorporating sibling interaction in the model means that the total variance of the phenotype becomes dependent on the genetic relationship of the subjects, resulting in different standardized estimates of A, D and E for MZ and DZ twins (33). This raises the question of generalizability of these findings to a population of non-genetically related individuals. It seems obvious that the heritability estimate will change, but it remains unclear in which direction. Two lines of reasoning can be put forward. As the models without the sibling interaction factor provide a heritability estimate independent from genetic relationship, one could argue that this estimate also applies to a population of non-genetically related individuals. In this case, the heritability estimate drops to 18%. However, ignoring the sibling interaction factor may be a simplification of what happens in reality. Looking at the models incorporating a negative sibling interaction, it can be seen that the heritability estimate increases along with decreasing genetic relationship (55% for MZ versus 68% for DZ). Following this trend, one can argue that the heritability estimate would further increase if the genetic relationship diminishes. In this case, the heritability estimate in a population of singletons is expected to be even higher than the 68% estimated in DZ twins

These results should be interpreted in the light of several methodological limitations. First, the sample consisted of female twin pairs in the general population. As there is some evidence that affective stress responses to daily life stressors differs for men and women (45), the findings may not generalise to males. Simultaneous analysis of male and female MZ and DZ pairs as well as opposite sex pairs will provide an opportunity to study quantitative and qualitative sex differences in affective stress response to daily life stressors. Second, this study focused on gene-environment interaction in daily life. However, one could argue that there is possibly also gene-environment correlation. Thus, the risk of being exposed to stresses in daily life may rise with increasing genetic liability to react stronger to stress. Kendler and colleagues showed that about one-third of the association between stressful life events and onset of major depression is explained by gene-environment correlation (46): subjects genetically predisposed to depression select themselves into high risk environments

Back to outline

(13, 47). In order to examine this issue, post-hoc analyses were carried out, using the co-twin control method (46, 48). If the correlation between the risk factor (exposure to daily life stressors) and the outcome (negative affect) is mediated by genetic factors predisposing to both the risk factor and (directly or indirectly) the outcome, the within DZ correlation should be higher than the within MZ correlation. The DZ correlation between minor daily stressors and negative affect equaled 0.52 ($p< 0.001$) and was indeed somewhat higher than the MZ correlation ($r_{MZ}= 0.47$, $p < 0.001$), suggesting the possibility of a small degree of genotype-environment correlation.

Third, the structural equation modeling procedure showed that our sample was under-powered to identify non-additive genetic effects in addition to additive genetic effects and sibling-interaction effects. Since samples in the order of ten-thousands of twin pairs are required to tease out these differences, and the bias in the results caused by an undetected genetic dominance is relatively small compared to the bias that results from rejecting sibling effect prior to evaluating the presence of genetic dominance (33), we have discussed the results of the more parsimonious AE-SI model.

To conclude, this was the first twin study ever to examine individual differences in affective response to small, daily life stress. As it was found that genetic factors explained most of the phenotypic variance, this study provided evidence for genotype-environment interaction in the flow of daily life. Some people are more sensitive to the effect of small stress because of their genes, and compensatory mechanisms may cause siblings in the same family to develop the trait in a directionally opposite fashion. The smaller, remaining part of the phenotypic variance was explained by individual-environmental factors.

Back to outline

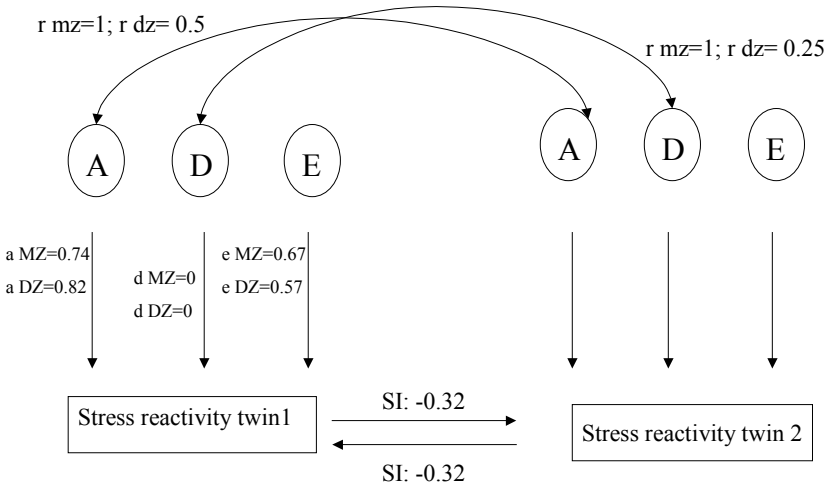
Table 1: Results of the univariate structural equation model fitting

Model	*-2LLn (df)*	*χ^2(df)*	*p*	*AIC*	*Model of comparison*	*$\Delta\chi^2$(Δdf)*	*p*	*A (95%C.I.)*	*D (95%C.I.)*	*E (95%C.I.)*	*SI (95%C.I.)*
saturated	461.98 (526)										
ADE-SI	475.65 (531)	13.67 (5)	0.02	3.67				MZ: 0.55 (0;0.63) DZ: 0.68 (0;0.77)	MZ: 0 (0;0.56) DZ: 0 (0;0.68)	MZ: 0.45 (0.36;0.56) DZ: 0.32 (0.23;0.48)	-0.32 (-0.42;-0.17)
AE-SI	475.65 (532)	13.67 (6)	0.03	1.67	ADE-SI	0 (1)	1	MZ: 0.55 (0.44;0.63) DZ: 0.68 (0.52;0.77)	-	MZ: 0.45 (0.36;0.56) DZ: 0.32 (0.23;0.48)	-0.32 (-0.42;-0.2)
E-SI	496.13 (533)	34.15 (7)	<.001	20.15	ADE-SI	20.48 (2)	<.001	-	-	MZ: 1 (1-1) DZ: 1 (1-1)	0.04 (-0.03;0.1)

A= Standardized Additive genetic effects; D= Standardized dominant genetic effects; E=Standardized individual-specific environmental effects; SI=sibling interaction; -2LL=minus twice the log likelihood of the raw data.; χ^2(df) is the difference in -2LL (df) of each model and the saturated model; AIC=χ^2-2df; $\Delta\chi^2$(df)=Likelihood ratio fit statistic, difference in χ^2(df) between 2 nested models (model of comparison is indicated in column 6); df =degrees of freedom; (95%CI)= 95% confidence interval of the estimate.

Back to outline

Figure 1 : Path diagram with the estimates of the AE-SI model



Note: The phenotypic variance of stress reactivity in each twin is determined by additive genetic factors (A), dominance genetic factors (D), individual-specific environmental factors (E) and sibling interaction effects (SI). Path estimates (a, d, e), are the standardized regression coefficients after the effects of SI on the total variance have been accounted for. The square of these estimates represent the proportion of variance they account for.

Back to outline

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Back to outline

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Back to outline

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Back to outline

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Back to outline

Chapter 9:

Epilogue

Back to outline

9.1. Summary and research findings

Heritability estimates of intelligence in twins: effect of chorion type

To examine to what degree individual differences in cognitive ability can be explained by genetic and/or environmental factors, structural equation modeling was applied to IQ data, gathered with the WISC-R in a large sample of young twins (270 MZ and 181 DZ). Results showed high heritability for almost all specific cognitive abilities as well as for general cognitive ability. The heritability of total IQ was estimated at 83%, with 95% C.I. ranging from 79 to 86%. These heritability estimates are in agreement with previous twin studies conducted in samples of the same age (Boomsma, 1993; Fulker and Cardon, 1993).

The second aim of this paper was to examine the effect of chorion type on the heritability estimates. As described in the introduction of this thesis, in most twin studies MZ twins are treated as a homogeneous group although this might not be the case. Therefore, structural equation modeling was applied to determine the influence of chorion type on IQ heritability estimates in 451 twin pairs of known zygosity and chorion type (175 MZ-MC, 95 MZ-DC and 181 same-sexed DZ). A significant effect of chorion type was found on two subscales, Arithmetic and Vocabulary. The effects accounted for respectively 14% and 10% of the total phenotypic variance. This result suggests that chorion type could be a confounding factor in a traditional twin study. The composition of the MZ group seems to be important for the accuracy of the heritability estimates, especially when the trait is supposed to be under the influence of chorion type. However, it must be mentioned that the chorion effects were found on only two subscales, and not on total IQ measures. Even more, the reported effects were small (10 and 14%) with confidence intervals close to zero. Although this study showed that the influence of chorion type on general cognitive ability was minimal, it is recommended to investigate possible chorion effects on the phenotype before treating MZ twins as one homogeneous group.

Child psychopathology and lower cognitive ability: a general population twin study of the causes of association.

Previous studies have shown that the association between measures of cognitive ability and psychopathology is stable over time and over different populations. However, the earliest origin of this association appears to be in childhood. Childhood psychopathology (CP) and lower cognitive ability (CA) may be associated because one is a risk factor for the other,

Back to outline

and/or because both are determined by a third cause. In this paper, the latter hypothesis is being studied. As univariate model fitting on both general cognitive ability and childhood psychopathology showed that both phenotypes were influenced by genetic and environmental factors, it was hypothesized that part of the observed association was caused by the same genes and/or environments. To test this hypothesis, the IQ data was combined with data on behaviour collected in the same twins (CBCL data). Results of the bivariate model fitting showed that, although the association between CA and CP itself was rather small (-0.19), genetic factors accounted for 84% of the observed association. This indicated that in children three different genetic factors exist that (1) solely influence the liability for CP, (2) only affect CA and (3) influence both CP and CA. This finding has implications for both clinical settings and research strategies. First, it may be useful for mental health professionals to be aware of the fact that a child with lower cognitive ability has a higher risk, regardless of the direction of causality, for psychopathology and vice versa. Given the existence of diagnostic overshadowing (i.e. the tendency to minimize or misdiagnose psychiatric disorders in the presence of low cognitive ability), this should be a special point of attention. Early identification of intellectual deficits among pre-schoolers may help to prevent later school difficulties and severe psychopathology. And second, the knowledge that there may be genes that influence CP as well as CA can help the search for specific genes in comorbid samples.

Deconstructing the familiality of the emotive component of psychotic experiences in the general population

Subclinical psychotic experiences, or “schizotypy”, are considered to be the phenotypic expression of the familial liability to schizophrenia. Schizotypy is a multidimensional construct consisting of at least 2 factors: positive psychotic experiences and negative psychotic experiences. Previous general population twin studies have already shown that genetic factors play a role in these dimensions of schizotypy. However, all previous research has focused on the familiality of occurrence and frequency of these subclinical psychotic experiences. Nevertheless, it has been suggested that it is not only the frequency, but also the associated distress that is important in the development of clinical need and patient status. Therefore, this paper examined i) the familiality of distress associated with positive and negative subclinical psychotic symptoms and ii) whether the distress was specific to the dimension or represented a general underlying distress factor.

Distress was measured in a large female general population twin sample, using the Community Assessment of Psychic Experiences questionnaire. Univariate structural equation

Back to outline

modeling revealed that the familiality of both distress related to positive subclinical psychotic symptoms and distress related to negative subclinical psychotic symptoms was based on a moderate genetic factor (not on common environmental factors). In addition, bivariate structural equation modeling demonstrated that the genetic and unique environmental factors influencing both phenotypes were correlated. Thus, the risk of becoming a patient seems to be based on the individual genetic vulnerability to feel distressed when experiencing a subclinical psychosis. Given these results, it is attractive to hypothesize that the nature of the psychotic experience (positive, negative, depressive…) may determine what kind of psychiatric illness is developed, while the risk of actually developing the psychiatric illness is, amongst others, based on the vulnerability to feel distressed by that symptom.

Electronic monitoring of salivary cortisol sampling compliance in daily life

A phenotype can be studied in experimental conditions or in real life. The environment can be perfectly controlled in an experiment, but in doing so, the generalisation of these results to circumstances outside the experiment becomes questionable. After all, most phenotypes and those in psychiatry in particular, are embedded in the context of daily life. Phenotypes such as depressed mood or psychotic symptoms, occur during daily activities while subjects are interacting with the persons and the world around them (Delespaul, 1995; deVries, 1992). Even more, it is plausible to suppose that such phenotypes are triggered or blocked by experiences in the environment. Therefore real life research gathers unique data, which is a valuable and important addition to data collected in the laboratory. Naturalistic research methods, such as the experience sampling method, have been developed to conduct research in the flow of daily life. As described in chapter 2, the Experience Sampling Method is a structured diary technique to assess subjects in their daily life environment, reducing biases in recall. In short, subjects receive a digital wristwatch that was programmed to emit a signal ("beep") at an unpredictable moment in each of ten 90-minute time blocks between 7:30 and 22:30, on five consecutive days. After each beep, subjects are supposed to take a saliva sample for cortisol determination and to fill out the ESM self-assessments forms previously handed to them, collecting reports of thoughts, current context (activity, persons present, location), appraisals of the current situation and mood. A possible drawback of this method is, however, the lack of control over participant' s compliance with the protocol, as self-reports are completed and samples taken without the researcher being present. Protocol compliance is crucial for the reliability and validity of the data, especially in the case of physiological parameters known to have a circadian rhythm or to be reactive to environmental stimuli, such

Back to outline

as saliva cortisol. This paper, therefore, investigated compliance with this Experience Sampling protocol for salivary cortisol in a sample of twenty female twin pairs and nineteen of their sisters. Results showed that compliance was satisfactory: 81% of all samples were accurately timed. In addition, inclusion of the non-compliant samples did not bias the cortisol profile. These results indirectly supported the validity of momentary self-report data (e.g. about mood or context) obtained with this intensive, random time sampling protocol.

A momentary assessment study of the relationship between affective and neuroendocrine stress responses in daily life

It has been suggested that the accumulation of stress-induced cortisol responses, which is thought to increase the risk for mental health outcomes such as depression, is mediated by the tendency to experience negative emotions in the face of stress. Momentary assessment methodology was used to measure stress as ongoing activity related stress and examine the relationships between stress on mood and HPA-activation. The Experience Sampling Method (ESM), a validated structured diary technique to assess stressors, mood and salivary cortisol at 10 random times daily for 5 consecutive days, was used in a general population sample of 556 women. Multilevel analysis was applied to investigate associations between ongoing stress, mood (negative affect, positive affect, agitation) and cortisol levels.
Minor daily life stress was associated with decreased positive affect, increased negative affect and increased agitation, but only negative affect was independently associated with increased cortisol levels. In addition, negative affect mediated part of the effect of stress on cortisol. The tendency to experience negative affective states in the flow of daily life likely explains part of the pathway whereby cumulative stress-induced cortisol responses shape the risk for mental health disorders.

Genes making one feel blue in the flow of daily life: A momentary assessment study of gene-stress interaction.

As the previous paper showed that the stress-induced increase in negative affect regulated the individual sensitivity to minor daily life stress, this article investigated to what extent individual differences in affective reactivity to minor daily life stress can be explained by genetic and/or environmental factors. First, a measure of stress-sensitivity was calculated for each subject, representing the individual change in negative affect associated with the subjective appraisal of minor daily stress. Second, classical twin modeling was applied to this stress sensitivity measure to disentangle genetic and environmental influences. Results

Back to outline

showed that 60 to 70% of the phenotypic variance was explained by genetic factors, while specific environmental factors explained the rest. As the stress reactivity measure included the interaction between stressor and emotional response, the demonstration of a genetic influence on this dynamic relationship is indicative of gene-environment interaction. Genes make some people more sensitive for the negative effect of minor daily events on mood, which might be associated with a higher risk to develop stress related disorders such as depression.

9.2. Nature-Nurture

The studies described in this thesis are all based upon the classical twin methodology. As mentioned in the introduction, a twin study is an ideal design to disentangle genetic and environmental influences on the phenotype being studied. However, the debate about nurture-nature is an ancient debate that is not to be expected to be resolved within the next years. Although the conclusive answers have not yet been found, progress has been made. During the last decade, a broader perspective on nature-nurture has been developed. In fact, the papers gathered in this thesis are a reflection of different views on nature-nurture and are illustrative of different approaches on this nature-nature debate.

A first approach is based on the assumption that genes and environment have an independent effect on the phenotype and is aimed at estimating the influence of genetic and environmental factors on the phenotype (see chapter 3). This kind of research has led to substantial changes in the way of thinking about determinants of health and disease. During the past decade, a shift has taken place from strict environmental explanations to a more balanced view that incorporates the importance of genes (Boomsma et al., 2002), for example in autism (Bailey et al., 1995; Bespalova and Buxbaum, 2003; Le Couteur et al., 1996). Furthermore, this classical approach also gives the opportunity to examine the influence of sex, age or other variables on the heritability estimates of the phenotype.

Another twin approach is aimed at identifying causes of co-morbidity between two or more phenotypes (see chapter 4 and chapter 5). This multivariate approach establishes the extent to which the clustering phenotypes share common genetic and environmental factors. For example, depression and generalized anxiety disorder are found to co-occur more often than expected by chance. Using a bivariate twin design, Kendler et al. (Kendler, 1996; Kendler et al., 1992) showed that the liability to both disorders was influenced by the same genetic factors. The co-morbidity of depression and anxiety disorder might be even an example of

Back to outline

genetic pleiotropy, i.e. a single genetic mutation explains (apparently) different disorders (Gorwood, 2004). More precisely, it is suggested that the gene coding for the serotonin transporter might be involved in the genetic vulnerability to both disorders.
During the last decade, the view on nature-nurture has become broader. The concepts of genotype-environment correlation and genotype-environment interaction have been introduced (see chapter 1), changing the way of thinking about environment and genes. The environment is not a random context in which the individual lives, but is partly created by that individual for genetic reasons (genotype-environment correlation). In addition, genetic factors affect the way the individual interact with the environment. Sensitivity to the environment is partly determined by genetic make-up (genotype-environment interaction). Chapter 8 focused on genotype-environment interaction and introduced a new way to investigate this. Using an experience sampling design (validated in chapter 6), a unique measure of the individual sensitivity for an environmental factor was applied (described in chapter 7). Classical twin modeling was then applied to this sensitivity-measure to examine whether genetic factors moderated the individual sensitivity to this specific environmental factor. This is a novel and attractive way to look for genotype-environment interaction without the necessity for knowledge about specific genes involved.

9.3. Nature-nurture in general cognitive ability

General cognitive ability is one of the oldest topics in behaviour genetics research. Most studies are based on IQ-tests (intelligence quotient tests) such as the Wechsler Intelligence Scale for Children-Revised (WISC-R) or the adult version, the Wechsler Adults Intelligence Scale (WAIS). These tests assess several cognitive abilities (such as specific verbal abilities) and result in a measure of general cognitive ability. It is now well established that genetic factors are explaining the major part of the individual differences in cognitive ability (see chapter 3 in which heritability of total IQ was estimated at 83%). Furthermore, our results indicated that the effect of chorionicity on general cognitive ability was minimal (although a small effect of chorion type was found on two subscales: Arithmetic and Vocabulary). In addition, reviews combining results from different studies have shown that the impact of genetic factors on general cognitive ability increases along with age (Boomsma, 1993; Plomin et al., 2000). It has been stated that genetic factors explain about 20% of the variance of general cognitive ability in early childhood. The impact of genes increases to about 40% in

Back to outline

later childhood and to 60% or even more in adulthood and later life. Common environmental factors are supposed to play a role only in early childhood, after then they fade away almost completely.

As general cognitive ability is one of the most inherited dimensions of behaviour and as it has been shown that the largest part of the genetic influences are additive, general cognitive ability should be a reasonable candidate for molecular genetic research (Plomin, 1999; Plomin et al., 2000). A lot of effort has been made to locate and identify related genes, but convincing evidence is still missing. Several explanations are possible. First, it is more likely that many genes with small effects contribute to the genetic variance rather than only one single gene accounting for all the genetic variance. This makes it harder to locate and identify associated genes. Molecular genetic strategies that can detect genes of small effect are therefore needed. Second, as described in the introduction of this thesis, genetic and environmental factors can correlate and interact. Several studies on general cognitive ability provide evidence for genotype-environment correlation, while genotype-environment interaction seems to be less likely (see (Plomin et al., 2000). People create their own intellectual environment partly based on their genetic make-up. This might also explain why the impact of genetic factors increases with age. It is possible that relatively small genetic effects snowball during development, creating larger and larger genetic phenotypic effects (Plomin et al., 2000). Along with age, intellectual experiences become more and more self-directed. People with a genetic predisposition towards high general cognitive ability will look for environments stimulating their cognitive abilities. While people without this genetic predisposition, will have this tendency to a lesser extent. This behaviour does not only reflect the genetic differences between people, but also enlarge them.

9.4. Nature-Nurture in daily life research

A great deal of this research is focused at (dis)stress. This is not surprising because stress has become inevitable in our dynamic Western society and the devastating impact of stress on general health becomes more and more clear. According to the World Health Organization, stress is expected to be one of the major causes of dysfunction for the next years (WHO, 2001).

Earlier research mainly investigated the impact of major stressful life events on general health and provided abundant evidence for a causal relationship. Stressful life events such as severe

Back to outline

financial problems or death of a loved one, are associated with the onset of common mental disorders such as depression (Hammen, 2003; Lopes et al., 2003).
However, recently, it has been suggested that daily hassles might be more important to general well being. Although the impact of daily hassles may be smaller, they occur much more frequently and, can therefore have a substantial effect on general health. Indeed, it has been demonstrated that minor stressors in daily life are associated with decreased psychological well-being and increased somatic symptomatology, and even with stress-related disorders such as depression and psychosis (DeLongis et al., 1988; Myin-Germeys et al., 2003; Stone et al., 1993; van Eck et al., 1998). Everyday life stress is thus a risk factor for decreased mental health. However, not everyone reacts in the same way to these minor stressors. Why do some people develop (psycho) pathology and others do not, when exposed to the same risk factor? Chapter 8 showed that genetic as well as environmental factors play a role in determining the individual sensitivity for minor daily hassles. Although studies have shown that an environmental factor, such as social support, is important in moderating the effect of stress on general health (Furukawa et al., 1999; Hammen, 2003; Shields, 2004), the findings of our study showed that genetic factors explained most of the individual differences in stress sensitivity. Up to 70% of the individual differences are explained by genetic factors. This finding not only confirmed earlier reports (Caspi et al., 2003; Kendler et al., 1995), but also extended them in an important way. After all, this thesis did not focus on major stressful life events, but explored the effect of minor stress as measured in daily life. By showing that genes and environment also interact in the flow of daily life, ecological validity was added to the concept of genotype-environment interaction.

9.5. Implications and directions for future research

The main findings from this thesis have several implications.
First, it was shown that minor daily stress was associated with changes in mood and in cortisol secretion (see chapter 7). Minor daily life stress has an impact on general health, which confirmed previous studies. People might develop psychological and physical complaints because of their sensitivity to minor stressors happening in everyday life. This implies that persons at risk could be identified based on their individual level of stress sensitivity. In addition, the findings from chapter 6 showed that the Experience Sample Method is a valid way to inventory such minor daily stressors and to examine subjective

Back to outline

reactions to these stressors. Furthermore, it was shown that the vulnerability for such stressors is based on genetic and environmental factors (see chapter 8). Consequently, identifying and modifying these factors will have an effect on the individual level of stress sensitivity, and thus also on the individual state of well-being. It is remarkable to notice that the greater part of research focuses on locating and identifying related genes, and much less research is aimed at identifying the associated environmental factors. `Environmental research` seems to be treated as a kind of stepchild. Environmental factors are recognized as being of `some importance`, but apart from that they are almost completely ignored. This field of environmental research is a challenge for future years (next to advanced genetic research of course). Just as genetic studies consider candidate genes, there may be candidate environments derived from theoretical mechanisms of aetiology. These environments can vary from situational to persistent, and from subtle to overt, from phenotype-specific to nonspecific and from phenotype-promoting to phenotype-inhibiting (Gunzerath and Goldman, 2003).

Second, this research focused at stress sensitivity in daily life. Minor stress is inevitable in daily life and people react to it, sometimes even without being conscious of it. However, people differ in the way they respond to stress, some being more susceptible to its effects than others (see chapter 8). In addition, the way in which people respond to stress has an impact on general health. It seems obvious that someone who is more sensitive to the effect of minor daily stress will experience a greater effect on his health than someone who is less sensitive. This point of view leaves behind the categorical view of ‘effect versus non-effect’ or in medical terms `sick versus non-sick`, and introduces a `continuum idea` into psychiatric research. According to this idea, stress-related symptoms are rather frequent in subjects from the general population (and thus not only present in subjects identified as `cases`) and in addition, they lie on a symptomatic continuum between `normal`, `symptom free` subjects and subjects diagnosed with a stress-related disorder. The latter display symptoms that are quantitatively, but not qualitatively, different from symptoms displayed by subjects without the disorder. Such a continuum view is already well accepted for physical characteristics like blood pressure or glucose tolerance, and evidence is growing showing that also psychological characteristics like psychotic experiences (such as hallucinations and delusions) and depressive symptoms are continuously distributed in the general population (Johns and van Os, 2001; Kendler and Gardner, 1998; van Os, 2003).

Third, the findings from this research can help in the search for genes associated with depression. In contrast with genetic studies on schizophrenia that have identified and

Back to outline

replicated seven susceptibility genes (Harrison and Owen, 2003; Harrison and Weinberger, 2004), genetic studies on depression are less convincing. Morley and colleagues (Morley et al., 2004) reported a number of identified candidate genes for depression, each of which increased the risk for mood disorders two or three fold. However, replications are still missing. This limited success raised questions concerning the definition of genetically relevant phenotypes, and it has been proposed to improve the phenotypic definition by introducing endophenotypes (Hasler et al., 2004). Endophenotypes represents simpler clues to the genetic basis of the disorder than the complex disorder itself, which increases the chance of success of finding associated genes. Criteria useful for identification of potential endophenotypes are (Gottesman and Gould, 2003):

(1) the endophenotype is associated with illness/disorder in the population
(2) the endophenotype can be inherited
(3) the endophenotype is manifest in an individual whether or not the illness/disorder is active
(4) the endophenotype and illness/disorder co-segregate within families.

As recently suggested by Hasler (Hasler et al., 2004), increased stress sensitivity can function as an endophenotype for depression. Our findings support this, by giving i) indirect evidence for criteria 1 and 3 and ii) direct evidence for criterion 2. In addition, Hasler (Hasler et al., 2004) also proposed increased action of the hypothalamic-pituitary-adrenocortical axis as an endophenotype for depression. Data gathered in this research (i.e. salivary cortisol secretion as response to minor daily life stress) allows to investigate if indeed, this biological measure of stress sensitivity can serve as endophenotype.

Furthermore, this study provided evidence for genotype-environment interaction. Up to 70% of the individual differences in stress sensitivity are explained by genetic factors. As mentioned before, this finding confirmed and extended earlier findings (Kendler et al., 1995). Caspi (Caspi et al., 2003) actually identified a functional polymorphism in the promoter region of the serotonin transporter gene that moderated the influence of stressful life events on depression. A recent study by Foley et al. (Foley et al., 2004) confirmed earlier findings (Caspi et al., 2002) that variation in MAO-A activity was associated with variation in sensitivity for childhood adversity. The approach of incorporating environmental factors in the design of molecular studies seems to offer more potential in finding associated genes. Even more, it has been suggested that environmental factors (such as stress) can modify gene expression without changing the DNA sequence (Abdolmaleky et al., 2004). Such epigenetic processes increase the complexity of genomic responses by allowing short-term fine-tuning of

Back to outline

the genome and provide a mechanism for maintaining information about environmental exposures. Evidence is growing that epigenetic effects may play a role in complex diseases such schizophrenia and mood disorders (Abdolmaleky et al., 2004; Henikoff and Matzke, 1997; Peedicayil, 2003).

Fifth and last, the concepts of genotype-environment interaction and correlation have provoked a fundamental change in our thinking about genes. For a long time it was believed that genes were determinative for almost everything going from colour of eyes to intelligence and even mood. Although genes are directly and exclusively related to some phenotypes (for example, physical characteristics such as colour of eyes or hair, but also some single-gene disorders such as down syndrome), a lot of other phenotypes are more complex. They are influenced not only by several genes with small effect, but even more, these genes act indirectly through exposure and sensitivity to environmental factors. This perspective on genes has moved us far away from any kind of deterministic way of thinking. Even more, the environment came back in the picture again.

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Back to outline

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Back to outline

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Back to outline

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Back to outline

SUMMARY

The common theme of the papers gathered in this thesis is that they are all 'twin studies'. The goal of **chapter 1** is, therefore, to describe the context of twin studies. A brief introduction into the twin methodology is given, showing the unique advantage of twin studies in disentangling genetic and environmental influences on the phenotype being studied
The papers in this thesis are related to two projects, which are described in **chapter 2**. The first project examined general cognitive ability in a large sample of young twins. The second project was a longitudinal twin study, focusing at stress reactivity in the flow of daily life.
Chapter 3 examined to what degree individual differences in cognitive ability can be explained by genetic and/or environmental influences. Results showed high heritability for almost all specific cognitive abilities as well as for general cognitive ability. In addition, a small effect of the chorion type was found on two subscales. However, the reported effects were small with confidence intervals close to zero. It was, therefore, concluded that the influence of chorion type on general cognitive was minimal and maybe even negligible.
Chapter 4 examined the hypothesis that childhood psychopathology and lower cognitive ability are associated because both are determined by a third (genetic) cause. Results showed that, although the association itself was rather small (-0.19), genetic factors accounted for 84% of the observed association. This indicated that in children three different genetic factors exist that (1) solely influence the liability for childhood psychopathology, (2) only affect cognitive ability and (3) influence both childhood psychopathology and cognitive ability.
Chapter 5 examined i) the familiality of distress associated with positive and negative subclinical psychotic symptoms and ii) whether the distress was specific to the dimension or represented a general underlying distress factor. It was shown that the familiality of both distress related to positive subclinical psychotic symptoms and distress related to negative subclinical psychotic symptoms was based on a moderate genetic factor (not on common environmental factors). In addition, it was demonstrated that the genetic and unique environmental factors influencing both phenotypes were correlated. Thus, the risk of becoming a patient seems to be based on the individual genetic vulnerability to feel distressed when experiencing a subclinical psychosis. It is attractive to hypothesize that the nature of the psychotic experience (positive, negative, depressive…) may determine what kind of psychiatric illness is developed, while the risk of actually developing the psychiatric illness is, amongst others, based on the vulnerability to feel distressed by that symptom.

Back to outline

Chapter 6 investigated compliance with the Experience Sampling protocol as used in the study. The Experience Sampling Method is a structured diary technique to assess subjects in their daily life environment, reducing biases in recall. In short, subjects received a digital wristwatch that was programmed to emit a signal ("beep") at an unpredictable moment in each of ten 90-minute time blocks between 7:30 and 22:30, on five consecutive days. After each beep, subjects were supposed to take a saliva sample for cortisol determination and to fill out the ESM self-assessments. Results showed that compliance was satisfactory: 81% of all saliva samples were accurately timed. In addition, inclusion of the non-compliant samples did not bias the cortisol profile. These results indirectly supported the validity of momentary self-report data (e.g. about mood or context) obtained with this intensive, random time sampling protocol.

Chapter 7 examined the relationships between ongoing activity related stress, mood and HPA-activation, using momentary self-report data that was validated in the previous chapter. Minor daily life stress was associated with decreased positive affect, increased negative affect and increased agitation, but only negative affect was independently associated with increased cortisol levels. In addition, negative affect mediated part of the effect of stress on cortisol. The tendency to experience negative affective states in the flow of daily life likely explains part of the pathway whereby cumulative stress-induced cortisol responses shape the risk for mental health disorders.

As the previous chapter showed that the stress-induced increase in negative affect regulated the individual sensitivity to minor daily life stress, **chapter 8** investigated to what extent individual differences in affective reactivity to minor daily life stress can be explained by genetic and/or environmental factors. Results showed that 60 to 70% of the phenotypic variance was explained by genetic factors, while specific environmental factors explained the rest. As the stress reactivity measure included the interaction between stressor and emotional response, the demonstration of a genetic influence on this dynamic relationship is indicative of gene-environment interaction. Genes make some people more sensitive for the negative effect of minor daily events on mood, which might be associated with a higher risk to develop stress related disorders such as depression.

The results of this thesis are discussed in **chapter 9** and are placed in the context of the ancient debate about nature-nurture**.** In fact, the papers gathered in this thesis are a reflection of different views on nature-nurture and are illustrative of different approaches on this nature-nature debate. Furthermore, implications and directions for future research are given in this last chapter.

Back to outline

SAMENVATTING

Tweelingenstudies zijn een uitstekende manier om het belang van erfelijkheid en milieu te bestuderen als verklaring voor verschillen tussen personen in bepaalde kenmerken of eigenschappen. Immers, eeneiige tweelingen zijn genetisch identiek en twee-eiige tweelingen delen slechts de helft van hun genetisch materiaal. Een grotere gelijkenis bij eeneiige tweelingen t.o.v. twee-eiige tweelingen is bijgevolg een aanwijzing voor een genetische invloed (hoofdstuk 1).

Het eerste deel van dit proefschrift (hoofdstukken 3-4) toont aan dat genetische factoren een belangrijke rol spelen in cognitieve bekwaamheden en laat zien dat deze genetische factoren grotendeels aan de basis liggen van associaties tussen cognitie en mentaal welzijn.

Het tweede deel van dit proefschrift (hoofdstukken 5-8) focust op stress- gevoeligheid in het dagelijks leven. Kleine dagelijkse stress gaat gepaard met veranderingen in affect waarbij vooral de stijging in negatief affect geassocieerd wordt met een biologische stressreactie. Bij deze affectieve stressreactie blijken genetische factoren een belangrijke rol te spelen.

De bevindingen worden besproken binnen het kader van huidige en toekomstige ontwikkelingen op het veld van genetisch onderzoek, zoals de inclusie van omgevingsvariabelen (GxE correlatie en GxE interactie) en de introductie van endofenotypes (hoofdstuk 9).

Back to outline

DANKWOORD

Dit proefschrift toonde (hopelijk) aan dat genetische en omgevingsfactoren niet los van elkaar gezien kunnen worden in hun effect op het fenotype. Ook ik ben een product van genen en omgevingsinvloeden en ben dan ook dank verschuldigd aan beiden.

Vooreerst, mijn ouders, die mij een pak genen met diverse functies meegaven.
Papa, van jou heb ik dat streven naar persoonlijke ontwikkeling meegekregen. De nadruk die jij legt op het verder uitbouwen van de talenten die iemand heeft, heeft me van in het begin heel sterk voort geduwd. Dit streven, aangevuld met jouw niet aflatende interesse en kritische ondersteuning heeft ervoor gezorgd dat ik ook dit proefschrift met succes kon afronden. Dank daarvoor !
Mama, van jou heb ik meegekregen dat het leven meer is dan werken alleen. Jij hebt me leren genieten van het leven en zorgde ervoor dat ik op moeilijke momenten de relativiteit van dit werk kon inzien. Die gezellige en ontspannende time-outs waren nodig om de voorbije jaren gemotiveerd aan dit project te kunnen blijven werken. Hopelijk volgen er nog veel van die time-outs !
En Tom, ‘mijn broerken ‘, jij hebt ondertussen ook jouw weg gevonden.Veel succes in jouw loopbaan als ‘echte’ dokter en veel geluk met Karen !

Genen alleen zijn niet voldoende en invloeden vanuit de omgeving dragen zeer zeker in grote mate bij aan het afronden van dit proefschrift.
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Back to outline

Wetenschap is jouw drijfveer en ik ben je dankbaar dat ik in jouw slipstream mee mocht drijven de voorbije jaren.
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Beste Frieda, ik durf nauwelijks te schatten hoeveel uren je besteed hebt aan het nauwkeurig invoeren van alle data die wij verzameld hebben. Jouw werk is het als het ware het begin van mijn werk en je weet, goed begonnen is al half gewonnen. Dank.
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Back to outline

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Back to outline

Curriculum Vitae

Nele Els Jacobs werd geboren op 19 juli 1974 in Gent (Belgie). Na het afronden van de studie Latijn-Wiskunde aan de Sint-Gertrudishumaniora in Wetteren, studeerde zij (klinische) psychologie aan de Rijksuniversiteit Gent. In 1997 werkte zij als klinisch psychologe verbonden aan de afdeling Palliatieve Zorgen van het Sint-Lucas ziekenhuis te Gent. Algauw maakte zij de overstap van klinisch werk naar wetenschappelijk onderzoek en was zij als wetenschappelijk medewerkster verbonden aan de Katholieke Universiteit Leuven. Zij was verantwoordelijk voor een tweelingenproject gericht op het identificeren van risicofactoren voor mentale retardatie. Sinds einde 1999 is zij werkzaam als assistent in opleiding bij de vakgroep Psychiatrie en Neuropsychologie, sectie Sociale Psychiatrie, van de Universiteit Maastricht. Zij verrichtte onderzoek naar stressgevoeligheid in het dagelijks leven, waarvan onderliggend document het resultaat is. Tijdens deze periode deed zij ook klinisch werk op de SPD van de RIAGG Maastricht en was zij ook betrokken bij onderwijsactiviteiten bij de faculteit Geneeskunde, Universiteit Maastricht. In 2004 werd haar het Kootstra Fellowship toegekend. Vanaf 1 oktober 2005 gaat ze als universitair docent klinische psychologie werken bij de Open Universiteit Nederland.

Back to outline

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Back to outline

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- N. Jacobs, F. Rijsdijk, C. Derom, M. Danckaerts, E. Thiery, R. Derom, R. Vlietinck, J. van Os (2003): *Child Psychopathology and lower cognitive ability: a general population twin study of the causes of association.* Behavior Genetics, Vol 33, No 6, p 706

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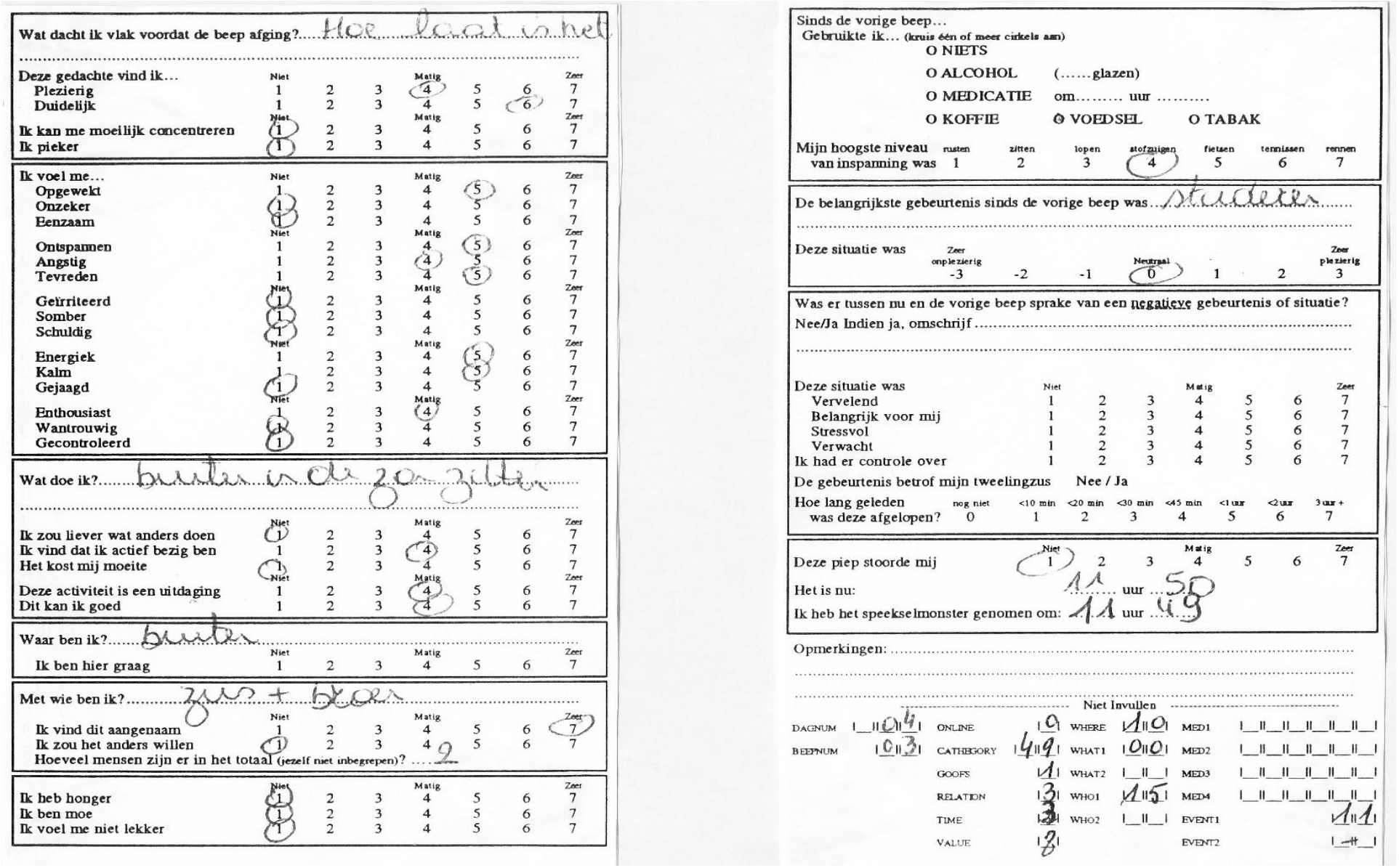
- N. Jacobs, F. Rijsdijk, C. Derom, M. Danckaerts, E. Thiery, R. Derom, R. Vlietinck, J. van Os: *Child Psychopathology and lower cognitive ability: a general population twin study of the causes of association.* World Journal of Biological Psychiatry (2001), vol. 2, Supplement 2

Back to outline

Appendix A



Wat dacht ik vlak voordat de beep afging? Hoe laat is het

Deze gedachte vind ik… (Niet 1 2 3 Matig 4 5 6 Zeer 7)
- Plezierig: 4
- Duidelijk: 6

Ik kan me moeilijk concentreren: 1
Ik pieker: 1

Ik voel me… (Niet 1 2 3 Matig 4 5 6 Zeer 7)
- Opgewekt: 5
- Onzeker: 1
- Eenzaam: 1
- Ontspannen: 5
- Angstig: 1
- Tevreden: 5
- Geïrriteerd: 1
- Somber: 1
- Schuldig: 1
- Energiek: 5
- Kalm: 5
- Gejaagd: 1
- Enthousiast: 4
- Wantrouwig: 1
- Gecontroleerd: 1

Wat doe ik? buiten in de zon zitten

Ik zou liever wat anders doen: 1
Ik vind dat ik actief bezig ben: 4
Het kost mij moeite: 1
Deze activiteit is een uitdaging: 4
Dit kan ik goed: 4

Waar ben ik? buiten

Ik ben hier graag: 4

Met wie ben ik? zus + broer

Ik vind dit aangenaam: 7
Ik zou het anders willen: 1
Hoeveel mensen zijn er in het totaal (jezelf niet inbegrepen)? 2

Ik heb honger: 1
Ik ben moe: 1
Ik voel me niet lekker: 1

Sinds de vorige beep…
Gebruikte ik… (kruis één of meer cirkels aan)
- O NIETS
- O ALCOHOL (……glazen)
- O MEDICATIE om…… uur ……
- O KOFFIE ⊗ VOEDSEL O TABAK

Mijn hoogste niveau van inspanning was (rusten 1, zitten 2, lopen 3, stofzuigen 4, fietsen 5, tennissen 6, rennen 7): 4

De belangrijkste gebeurtenis sinds de vorige beep was… studeren

Deze situatie was (Zeer onplezierig -3 -2 -1 Neutraal 0 1 2 Zeer plezierig 3): 0

Was er tussen nu en de vorige beep sprake van een negatieve gebeurtenis of situatie?
Nee/Ja Indien ja, omschrijf ……

Deze situatie was (Niet 1 2 3 Matig 4 5 6 Zeer 7)
- Vervelend: 1
- Belangrijk voor mij: 1
- Stressvol: 1
- Verwacht: 1

Ik had er controle over: 1
De gebeurtenis betrof mijn tweelingzus: Nee / Ja
Hoe lang geleden was deze afgelopen? (nog niet 0, <10 min 1, <20 min 2, <30 min 3, <45 min 4, <1 uur 5, <2 uur 6, 3 uur + 7): 0

Deze piep stoorde mij (Niet 1 2 3 Matig 4 5 6 Zeer 7): 1
Het is nu: 11 uur 50
Ik heb het speekselmonster genomen om: 11 uur 49

Opmerkingen: ……

Niet Invullen

DAGNUM	04	ONLINE	0	WHERE	10
BEEPNUM	03	CATHEGORY	49	WHAT1	00
		GOOFS	1	WHAT2	
		RELATION	3	WHO1	15
		TIME	3	WHO2	
		VALUE	3		

MED1	
MED2	
MED3	
MED4	
EVENT1	11
EVENT2	

Back to outline

! Appendix B

```
!Mx univariate script: Heritability Estimates of Intelligence in twins: effect of chorion type (chapter 3)
! TIQ - ACE-chorion model – variance covariance matrix

#define nvar 1                          ! define number of variables

Title Group 1: parameters of model
 Calculation Ngroups=4
 Begin Matrices;
  X Full nvar nvar Free                 ! a: additive genetic parameter
  Y Full nvar nvar Free                 ! c: common environment
  T Full nvar nvar Free                 ! ch: chorion parameter
  Z Full nvar nvar Free                 ! e: unique environmental parameter
  H Full 1 1                            ! scalar .5
 End Matrices;
 Matrix H .5
 Begin Algebra;
  A=X*X';                               ! a^2: additive genetic variance
  C=Y*Y';                               ! c^2: shared environmental variance
  B=T*T';                               ! ch^2: chorion variance
  E=Z*Z';                               ! e^2: unique environmental variance
  V=A+C+B+E;                            ! total variance
  P=A|C|B|E;                            ! put parameter estimates in one matrix
  S=P@V~;                               ! standardized parameter estimates
  J=A%V;                                ! gestandaardiseerde A
  K=C%V;                                ! gestandaardiseerde C
  L=B%V;                                ! gestandaardiseerde B
  M=E%V;                                ! gestandaardiseerde E
 End Algebra;
 Interval @95 J 1 1 1
 Interval @95 K 1 1 1
 Interval @95 L 1 1 1
 Interval @95 M 1 1 1
Start 1 all
BO -150 150 all
End

Title G2: MZ mc pairs
DATA NINPUT_VARS=2 NOBSERVATIONS=175
CMATRIX
  230.1443678
  170.0600000       183.1534975
LABELS TW1-N TW2-N
 Matrices = Group 1
 Covariances A+C+B+E | A+C+B _
                  A+C+B    | A+C+B+E ;
 Option Rsiduals
End

Title G3: MZ dc pairs
DATA NINPUT_VARS=2 NOBSERVATIONS=95
CMATRIX
 205.8024636
 178.4555431       230.0015677
```

Back to outline

```
 LABELS TW1-N TW2-N
 Matrices= Group 1
 Covariances A+C+B+E   | A+C _
                A+C           | A+C+B+E ;
 Option RSiduals
End

Title G4: DZss pairs
 DATA NINPUT_VARS=2 NOBSERVATIONS=181
CMATRIX
     228.7067526
    100.7873849       227.5735421
LABELS TW1-N TW2-N
 Matrices= Group 1
 Covariances A+C+B+E   | H@A+C _
                H@A+C      | A+C+B+E ;
 Option RSiduals
 Option NDecimals=4
Option TH=2
Option CInterval=95
Option Multiple
End

! test ACE-model (zonder chorion)
save acche.mxs
drop 3
end

! test AE-chorion model
get acche.mxs
drop 2
end

! test AE-model (zonder chorion)
drop 3
end

! test CE-chorion model
get acche.mxs
drop 1
end

! test CE model (zonder chorion)
drop 3
end

! test E-chorion model
get acche.mxs
drop 1 2
end

! test E-chorion
drop 3
end
output
```

Back to outline

! **Appendix B**

! Mx bivariate script: Child Psychopathology and lower cognitive ability (chapter 4)

! Genetic Cholesky Model op ruwe data (dus model met gemiddelden)
! Volledige model: ACE bij IQ en CBCL en alle 3 correlaties

```
#define nv 2

G1: Specify matrices
Data Calc NGroups=4
Begin Matrices;
G Lower nv nv Free                    ! A
B Lower nv nv Free                    ! C
F Lower nv nv Free                    ! E
M full 1 nv Free                      !means

H Full  1 1 Fix
End matrices;

Begin Algebra;
A=G*G'  ;
C=B*B'  ;
E=F*F'  ;
P=A+C+E ;
End Algebra;

SP G
 1
 2 3
SP B
 4
 5 6
SP F
 7
 8 9

 SP M   10 11

start .8 G 1 1  F 1 1   B 1 1
start .7 G 2 2  F 2 2   B 2 2
start -1 G 2 1  F 2 1   B 2 1

start 2 M 1 1
start 10 M 1 2

MA H .5
End

G2: MZ twin pairs
Data NInput_vars=11 NObservations=0
Missing =-999
RE File=mergesel.raw
Labels  id sexzyg zyg cbcl1 cbcl2 t1 v1 p1 t2 v2 p2
SELECT if zyg = 1 /
```

Back to outline

```
SELECT cbcl1 t1 cbcl2 t2 /
Matrices = group 1

Covariances A+C+E | A+C _
          A+C   | A+C+E /

Means M|M /

opt rs
End

G3: DZ twin pairs
Data NInput_vars=11 NObservations=0
Missing =-999
RE File=mergesel.raw
Labels  id sexzyg zyg cbcl1 cbcl2 t1 v1 p1 t2 v2 p2
SELECT if zyg = 2 /
SELECT cbcl1 t1 cbcl2 t2 /
Matrices = group 1


Covariances A+C+E   | H@A+C _
          H@A+C   | A+C+E /

Means M|M /
opt rs
End

G4: Standardized Solution
Calculation Group
Begin Matrices;
A com =A1
C com =C1
E com =E1
P com =P1
End Matrices;
Begin Algebra;
L=\stnd(A)| \stnd(C) | \stnd(E);          ! Genetic|C|E correlation
N=A%P | C%P | E%P ;                      ! h2=heritability/c2/e2

Y=\stnd(A);
X=\stnd(C);
Z=\stnd(E);

End algebra;

Interval @95 Y 2 1
Interval @95 X 2 1
Interval @95 Z 2 1

Options Multiple NDecimals=4
end
```

Back to outline

! **Appendix B**

! Mx univariate script: gene-stress interaction in daily life (chapter 8).
! Volledige model ADE Sibling Interaction

```
#DEFINE NV 1
G1: SPECIFY MATRICES
DATA CALC NGROUPS=5
BEGIN MATRICES;
X LOWER NV NV FREE   ! GENETIC PATH
Y LOWER NV NV FIX    ! D PATH
Z LOWER NV NV FREE                                  ! E PATH
M FULL   1 NV FREE                                  ! MEANS
H FULL  1 1 FIX
Q FULL  1 1 FIX
B FUL 2 2                                           !INTERACTION PARAMETER
I IDE 2 2
END MATRICES;
BEGIN ALGEBRA;
A=X*X'  ;
D=Y*Y'  ;
E=Z*Z'  ;
P=A+D+E ;
END ALGEBRA;
SP B
0 5
5 0
START 0.4 ALL
START -.3  B 2 1
START 3  M 1 1
MA H .5
MA Q .25
END

G2: COVARIANCE  FOR MZ PAIRS
DATA NINPUT_VARS=4 NOBSERVATIONS=0
MISSING =999
RE FILE=datadefn.raw
LABELS idtw vartrans1 vartrans2 zyg
SELECT IF ZYG = 1 /
SELECT vartrans1 vartrans2 /

MATRICES = GROUP 1
COVARIANCES        (I-B)~ *(A+D+E | A+D _
                    A+D   | A+D+E) *((I-B)~)' /

MEANS M|M /
OPT RS
END

G3: COVARIANCE  FOR DZ PAIRS
DATA NINPUT_VARS=4 NOBSERVATIONS=0
MISSING =999
RE FILE=datadefn.raw
LABELS  idtw vartrans1 vartrans2 zyg
```

Back to outline

```
SELECT IF ZYG = 2 /
SELECT  vartrans1 vartrans2 /
MATRICES = GROUP 1
COVARIANCES        (I-B)~ *(A+D+E          | H@A+Q@D _
                   H@A+Q@D | A+D+E) *((I-B)~)' /

MEANS M|M /
OPT RS IT=1000 ND=3
END

G4: STANDARDIZED SOLUTION for full ADE+i model MZ GROUP
CALCULATION GROUP
BEGIN MATRICES;
U SYMM 2 2 =%E2                                    !Expected MZ Cov Matrix
A COMP 1 1 =A1
D COMP 1 1 =D1
E COMP 1 1 =E1
B FULL 2 2 =B1
I IDEN 2 2
Z ZERO 1 1
R full 1 4
End Matrices;
MA R 1 1 1 1

BEGIN ALGEBRA;
S=\part(U,R);                                      !is MZ total variance
T=(I-B)~ *(A | A _ A | A) *((I-B)~)' ;             !A variance/cov incorporating Sib-Int
L=(I-B)~ *(D | D _ D | D) *((I-B)~)' ;             !D variance/cov incorporating Sib-Int
Y=(I-B)~ *(E | Z _ Z | E) *((I-B)~)' ;             !E variance/cov incorporating Sib-Int
P=\part(T,R);                                      ! Var A incorporating Sib-int
Q=\part(L,R);                                      ! Var D incorporating Sib-int
N=\part(Y,R);                                      ! Var E incorporating Sib-int
V=P%S | Q%S| N%S;                                  !Interaction-Corrected h2|d2|e2for MZ
END ALGEBRA;

OPTIONS NDECIMALS=4 rs
INTERVAL V 1 1  V 1 2 V 1 3
INTERVAL B 2 1
END

G5: STANDARDIZED SOLUTION for full ADE+i model DZ GROUP
CALCULATION GROUP
BEGIN MATRICES;
U SYMM 2 2 =%E3                                    !Expected DZ Cov Matrix
A COMP 1 1 =A1
D COMP 1 1 =D1
E COMP 1 1 =E1
B FULL 2 2 =B1
H FULL 1 1 =H1
Q FULL 1 1 =Q1
I IDEN   2 2
Z ZERO 1 1
R full 1 4
End Matrices;
MA R 1 1 1 1
```

Back to outline

```
BEGIN ALGEBRA;
S=\part(U,R);                                   !is DZ total variance
T=(I-B)~ *(A | H@A _ H@A | A) *((I-B)~)' ;      !A variance/cov incorporating Sib-Int
L=(I-B)~ *(D | Q@D _ Q@D | D) *((I-B)~)' ;      !D variance/cov incorporating Sib-Int
Y=(I-B)~ *(E | Z _ Z | E) *((I-B)~)' ;          !E variance/cov incorporating Sib-Int
P=\part(T,R);                                   ! Var A incorporating Sib-int
W=\part(L,R);                                   ! Var D incorporating Sib-int
N=\part(Y,R);                                   ! Var E incorporating Sib-int
V=P%S | W%S| N%S;                               !Interaction-Corrected h2|d2|e2for DZ
END ALGEBRA;

OPTIONS NDECIMALS=4 rs
 INTERVAL V 1 1  V 1 2 V 1 3
INTERVAL B 2 1
END
```

Back to outline