The ecogenetics of schizophrenia

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The ecogenetics of schizophrenia

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The ecogenetics of schizophrenia

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CHAPTER 1

Introduction

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Schizophrenia Research, 32, 127-135

1 Genetic epidemiology in relation to epidemiology

Epidemiology can be seen as the basic science of investigating diseases in human populations. Its development was greatly accelerated by the finding of an association between smoking and lung cancer by Bradford Hill and Richard Doll. Increasingly refined methods have been devised to measure the impact of environmental factors on disease, and to use this knowledge in the treatment and prevention of illness.

Later developments in epidemiology have focused on the study of the interrelationship of several risk factors in the causation of disease. Rothman (Rothman 1986) defined a *sufficient cause* of disease as "a set of minimal conditions and events that inevitably produce disease.....". In many illnesses, including psychiatric disorders, genes form part of the component factors of the sufficient cause. For example, while smoking increases the risk for lung cancer, many heavy smokers do not develop cancer because of, for example, differences in genetically determined enzyme systems (Caporaso et al 1989). Another example is differences in the risk of cardiovascular disease due to genetically determined differences in the metabolism of dietary cholesterol (Goldstein et al 1989). Genetic epidemiology examines, amongst others, such interactions between environmental and genetic factors in the causation of disease. Progress in genetics is therefore shaping the future of epidemiology (Editorial 1996a).

2 The nature-versus-nurture debate in psychiatry

The notion that psychiatric disorders are the result of complex interactions between constitutional vulnerability and social precipitants has long held sway. In spite of its intuitive attractiveness as a theory, however, little effort was directed towards the

elucidation of nature-nurture interactions in psychiatric research and psychiatric clinical reasoning. Instead, there was, and continues to be to a degree, something of a stand-off between advocates of nature and champions of nurture (Van Os et al 1993).

Macro-environmental theories of disease aetiology have tended to dominate clinical psychiatry in continental Europe, whereas theories derived from genetic epidemiological research were less successful in penetrating the clinical domain and psychiatric training (Van Os et al 1993). One of the factors contributing to this state of affairs is, as one observer noted not so long ago, that genetic epidemiologists may be best described as "a self-contained group of research workers using complex statistical models understandable to this group alone" (Motulsky et al 1984). What also did not help was that workers in the field of molecular genetics have been observed to put forward models that seemed rather narrow and deterministic, thus fuelling the nature-versus-nurture debate.

It is now increasingly recognized, however, that collaboration between clinicians, social scientists and geneticists is necessary in order to study the ways in which genes influence environments and vice versa. Stressing this view, Reiss and colleagues (Reiss et al 1991) spoke of psychiatric genetics as "an unheralded window on the environment". Those wishing to study nurture need to control for genetic "noise", and those that wish to study nature need to control for environmental factors. The influence of genes, at least as far as complex psychiatric disorders are concerned, is probabilistic rather than deterministic, and complicated interactive patterns between genes and environments may be the rule rather than the exception.

3 Principles of genetic epidemiology relevant to clinical psychiatry

3.1 General issues

It is helpful to know what psychiatric genetic epidemiology is not. Some of the most common misconceptions about genetic epidemiology are listed in Table 1 to guide the reader. The more relevant aspects of genetic epidemiology in relation to clinical psychiatry are embodied in the following two questions, the first descriptive and the second analytic: i) do psychiatric disorders tend to cluster in families, and ii) what are the respective and interactive roles of genes and environment in making members of the same family *similar* to each other on the one hand, and *different* to each other on the other? Genetic epidemiology addresses these issues by epidemiological analysis of disease occurrence in groups of genetically related individuals. In doing so, it uses a set of methods and statistical approaches for the study of genetic factors, the development of which started in the second half of the 19th century (Khoury et al 1993). These approaches allow investigators to test

predictions about patterns of gene transmission from parents to offspring, and to compare the likelihood of different models of the relative contributions of genes, environments and their interactions to a particular characteristic. More complete and technical descriptions of these issues can be found elsewhere (Khoury et al 1993; McGuffin et al 1994; Plomin et al 1997).

Table 1. *Common misconceptions in clinical psychiatry about genetic epidemiology*

Misconception	Remarks
Genetic research is pessimistic: genes determine one's fate.	There are no simple, one-to-one gene-behaviour pathways. Behavioural outcomes of genes are subject to a multitude of environmental, stochastic and epigenetic influences that cannot be predicted. Therefore, genetic effects on behaviour are probabilistic, not deterministic. Genes code for proteins, not behaviour.
Genes that contribute to psychiatric disease must be bad genes	Genes that are involved in psychiatric disease are in all likelihood also common in the general population. Moreover, the same genetically influenced behaviour may increase the risk for some outcomes, but may serve as a protective factor for others. For example, behavioural inhibition is a risk factor for anxiety disorders (Biederman et al 1995), but a protective factor against antisocial behaviour (Cloninger et al 1996).
Genetic research is trying to identify the gene for schizophrenia so that selective abortions can be recommended.	Genetic influences on psychiatric disorders probably involve many common genes, each of small effect, and none of which is either necessary or sufficient to produce disease. However, identification of genes will increase knowledge on interactions with environmental risk factors, and on the pathophysiology and treatment of disease. Prevention of genotypes can never be acceptable in psychiatry. However, prevention of phenotypes by targeting environmental risk factors that interact with genotypes is.
Genetic research is ignoring the role of the environment	Among the most important findings of genetic epidemiology is scientific proof that the environment contributes in an important way to behavioural outcomes. Because it uses methods that enable researchers to filter out genetic "noise", genetic epidemiology is particularly well suited to examine environmental influences. Genetic epidemiology and behavioural genetics offer "an unheralded window on the environment" (Reiss et al 1991).

Genetic aetiology is incompatible with effective therapeutic intervention

1. In the case of genotype-environment interaction, effective treatment of genetic defect is possible through environmental manipulation. For example phenylalanine-free diet in the treatment of phenylketonuria.
  2. Potential of identification of individuals at risk and early treatment that may improve long-term prognosis.
  3. Potential of discovery of effective treatments through insight in the way disease genes produce proteins and/or control other genes.
  4. Potential of gene-therapy.
-

In epidemiological terms, any genetic effect can be considered a risk factor. The unique feature of genetic epidemiology is that the genetic risk factors can be studied without direct measurement, by examining familial clustering of associated traits. These can be physically observable traits on the one hand (exophenotypes or simply phenotypes), such as motor tics or delusions, or measurable biological traits (endophenotypes) on the other, such as cerebral ventricle size on MRI images or other traits described by Gottesman (Gottesman 1991) as "*data not available to the naked eye that are intermediate to the phenotype and the genotype...*". Thus, familial clustering of a disease phenotype can be demonstrated by studying the rate of disorder in the first degree relatives of individuals with the disease (the probands), and comparing this with the rate of disease in first degree relatives of controls. Twin studies, adoption studies and special types of family studies can subsequently be used to disentangle the respective roles of genes and environment in the causation of familial aggregation (Table 2). The principle of these studies involves comparisons in which either genetic similarity is varied randomly while environmental similarity is held constant, or vice versa. This can be accomplished through knowledge that certain family relationships are not as similar genetically or environmentally as others. For example, genetic effects can be traced by examining the degree of resemblance of reared apart genetically identical twins (who share the same genes but not the environment), whereas familial environmental effects can be traced by examining the degree of resemblance of non-genetically related adoptive relatives (who share the same environment but no genes). The strength of this approach is that statements can be made on the relative contributions of genes and environments through inference, without the need of direct measurement. This is not to say that direct measurement of genes and environments is not desirable. As we shall see later, measurement of specific factors can, where possible, yield important clues to patterns of interactions between genes and environment.

Table 2. *Most common types of studies in epidemiological and molecular genetics.*

	Aims	Remarks.
FAMILY STUDIES	1. identification of familial aggregation of diseases 2. study of familial syndromal overlap 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 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 twins, which may contribute to the greater degree of phenotypic resemblance of MZ twins, and may inflate the associated heritability estimates. Furthermore, there is evidence 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 (Reiss et al 1991).
-twins reared apart	direct estimate of the magnitude of heritable effects	
-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 2. identification of correlates of disorder	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).
-offspring discordant twins	confirmation of unexpressed genotypes	
		The generalisability of results obtained from twin studies to the general population is uncertain.
ADOPTION STUDIES		
-adoptive 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.
-adoptive 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.
-adoptive's family study	resemblance between affected adoptees and biological parents indicates shared heridity	Selective placement by adoption organizations according to ethnic, religious, socioeconomic 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.

-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.

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 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.

With the advent of molecular genetic techniques, it has now also become possible to examine genetic variation at the molecular level, and to relate these to particular phenotypic outcomes. Molecular genetic studies are based on the principle that disease occurrence can be linked to a genetic marker of some sort. An *allele* is one of the alternative versions of a gene (such as the different alleles of the gene determining the color of the seeds of Mendel's pea plants). If a gene has two or more alleles with a frequency of at least 1% in the population, it is customary to speak of a *polymorphism*. The *locus* is the position of the gene on the chromosome, which may thus be occupied by different alleles in different individuals. Knowledge about the relative frequencies of the different alleles of such a polymorphism in diseased and non-diseased individuals in the same family can yield information about the location of genes that influence the risk of disease. For example, if in a large family with a high rate of disease X over several successive generations most of the diseased individuals have allele A of gene Y, whereas most of the non-diseased have allele B, it can be inferred that the locus of the disease gene is apparently so tightly linked (ie. is very nearby) to the locus of the gene Y that their alleles (allele A and B for gene Y and the disease and non-disease alleles for the disease gene) are transmitted together (or co-segregate) most of the time in this family. Had this tight linkage not existed, then the alleles of gene Y and the disease gene would have been subject to the normal process of *recombination*, whereby new combinations of paternal and maternal alleles would have been created, and individuals with the disease would have displayed allele A as frequently as allele B. If linkage has been established and the site of gene Y is known, it can be inferred that the disease gene must be in close proximity to the locus of gene Y on its particular chromosome. This chromosomal region can then be characterised further with molecular genetic techniques to identify the genetic defect.

3.2 Sources of phenotypic variance in psychiatry

3.2.1 Genes and environment

Phenotypes are the result of the influence of genotype and environment. Phenotypes may be discrete (for example affected or not affected with hypertension) or continuous (for example blood pressure). In the case of a continuous phenotype, genetic and environmental influences are expressed as the degree to which phenotypic deviations from the mean (phenotypic variance) for some character in a population are a function of these environmental and genetic deviations. In the case of discrete phenotypes, an underlying continuously distributed disease *liability* is assumed to exist (a quantitative measure of underlying vulnerability), the variance of which can also be modelled as a function of environmental and genetic deviations.

Environmental and genetic influences on the phenotype can be divided into familial and non-familial components. Since the genes of an individual are always derived from the parents, related individuals do not have DNA unique to that person in the family (ie genetic factors are familial). However, environmental factors may be either shared with other relatives, or may be unique to the individual. For example, all the children in a family living together may share the same crowded housing conditions; these same children, however, may all go to different schools whose environments they do not share with their siblings. These familial and non-familial environmental and genetic influences on the phenotype are summarized in figure 1.

Fig. 1 Contribution to the phenotype

Genetic and environmental influences predict both differences and similarities between relatives. Consider full siblings living in the same family. Such siblings who share on average 50% of their genes should resemble each other more on genetically influenced traits than individuals picked at random from the general population, who do not share the same genetic material. However, they should also *differ* from each other more than

genetically identical twins, because full siblings also differ in 50% of their genes whereas identical twins share 100% of their genetic material. Similarly, genetic epidemiology predicts that individuals in the same family should resemble each other because they share a multitude of environmental influences (shared familial environment), such as parental social class, housing, parental illness and parental rearing practices. However, environmental influences on phenotypic variance also operate to make siblings *different* from each other, to the extent that the environmental factors are *not* shared with the sibling (non-shared environment) (Plomin et al 1987). These include, for example, differential parental treatment, experiences at school, peer influences, and personal illness or accidents.

3.2.2 Familial and non-familial contributions to the psychiatric phenotype

Which of the possible familial and non-familial influences on phenotypic variance are relevant in psychiatry? Familial influences, as we have seen in figure 1, can be either genetic or environmental. Numerous twin and adoption studies have established that genes influence all types of psychopathology, as will be discussed in more detail below. Secondly, and for practising psychiatrists possibly most importantly, one of the most consistent findings of the combined research is that the shared family environment accounts for a small proportion of the variance of psychiatric phenotypes such as schizophrenia, depression, bipolar disorder and aspects of normal and abnormal personality (Kendler et al 1992c; Kety et al 1994; Loehlin 1992; McGuffin et al 1984a; McGuffin et al 1994; McGuffin et al 1993; Saudino et al 1996), with the exception of i) juvenile anti-social behavior, where twin studies have demonstrated substantial shared environmental influence (Rowe 1983; Rowe 1986), and ii) shared environments which are extreme in some way, as the influence of these has been less well researched (Goodman 1991). The lack of influence of the shared environment does not indicate that the role of the environment is not important. On the contrary, research in the last decades has uncovered substantial environmental influences. However, the factors that are important to personality and psychopathology are those factors that are *not* shared by members of the same family: the nonshared environment. In other words: family members *resemble* each other on measures of psychopathology primarily because of shared DNA, whereas the nonshared environment operates to make members of the same family *different* from each other in this respect (Plomin et al 1987).

3.2.3 The nonshared environment and psychopathology

The finding that the non-shared environment, and not the shared family environment, contributes to measures of psychopathology and personality development may seem counterintuitive at first for two reasons. First, familial aggregation of psychiatric disorders is thought by many to arise because of familial aggregation of environmental risk factors, such as patterns of parental interaction with the children, rather than genetic factors (Van Os et al 1993). However, this is unlikely to be true for most cases because, before anything else, it can be demonstrated mathematically that familial clustering of environmental risk factors can only account for a small proportion of familial clustering of psychiatric disease. This is because the effect sizes of any investigated early environmental risk factor for later psychiatric outcomes are far too small to be able to explain the high recurrence risks in first degree relatives such as siblings (Khoury et al 1988). In addition, as reviewed earlier, one of the most consistent findings in genetic epidemiology in the last decades has been that the shared family environment contributes little to the variance of psychiatric phenotypes.

Second, many psychiatric environmental macro-theories do not primarily predict differences between children *within* the same family. Rather, they predict differences *between* families. The findings from genetic epidemiology teach us that environmental theories should predict differences between siblings in the same family, because the nonshared environment acts to make children living in the same family different from each other. Given these facts, Pike and Plomin pointed out that, from the clinical point of view, it would appear important to focus routinely on the whole family rather than the family member with the clinical problem (Pike et al 1996). A recent study by Reiss and colleagues, called "genetic questions for environmental studies" validated this point. In a total of 708 families with at least two same-sexed adolescent siblings of different genetic relatedness (MZ twins, siblings, halfsiblings, genetically unrelated siblings in step families etc.), the authors examined psychiatric symptoms in the adolescents and patterns of interactions between the parents and children. They found that around 60% of the variance in adolescent antisocial behaviour and 40% of the variance in depressive symptoms could be accounted for by conflictual and negative parental behaviour *directed specifically* at the adolescent. Interestingly, dysfunctional parenting style directed specifically towards one sibling predicted *less* psychopathological outcome in the other (Reiss et al 1995). Thus, this study suggests that the parental behaviour that influences psychopathology in the children is of the nonshared type, that is, directed specifically at the child and not its siblings. Furthermore, dysfunctional parental interaction with one sibling may protect the other from developing psychopathology. Other studies have also found associations between differential parental treatment and maladjustment in adolescent siblings (Dunn et al 1990; Tejerina-Allen et al 1994).

Reiss and colleagues also reported that parents are much more likely to recount that they treat their children similarly than their children do. Children appear therefore much more sensitive to differential parental treatment. As such differential parental treatment may affect their psychological development and their mental health, this should obviously be a matter of concern for clinicians. It is unlikely that differential parental treatment is the only nonshared environmental factor that influences psychopathology. Many other factors may exert nonshared environmental effects on psychopathology. These may include any biological or social environmental risk factor such as drugs, pollutants, accidents, peer interactions and so on. Indeed, some nonshared environments may be unique to a single family. The important issue raised by the study by Reiss and colleagues is that environmental hypotheses generated by genetic epidemiology can actually be investigated and translated into clinically relevant matters. These and forthcoming studies are in the process to further unravel the relationship between the nonshared environment on the one hand, and psychological development and psychopathology on the other. They may eventually also yield clues to ways of prevention and intervention.

3.2.4 To what degree are measures of the family environment really environmental?

The above reported findings that differential parental treatment is associated with adolescent psychopathology requires cautious interpretation. Dimensions of parental interaction, such as parental warmth, parental control and parental negativity cannot merely be seen as environmental influences bestowed onto the child as passive recipient. Modern environmental theories view the environment as not only what happens to the child, but also what the child makes happen. Thus, parental responses to the child may be as much child-driven as they are parent-driven. Certain characteristics in the child, such as features of temperament or personality, may elicit certain parental responses.

The fact that characteristics in the child may influence parental response is important from the point of view of genetic epidemiology, because if some of these child characteristics are (partly) under genetic control, measures of parental interaction themselves will also show genetic effects (Plomin 1994). For example, a child scoring high on extraversion may elicit more controlling responses from its parents. If extraversion has a degree of genetic control (and the evidence indicates that it has (Loehlin 1992)), monozygotic twins will resemble each other more on extraversion than dizygotic twins, indicating a genetic effect. However, if we were to measure the perceptions of parental control in these same MZ and DZ twins, we would also find a genetic effect, because parental control is associated with extraversion. In other words: measures of the family environment such as dimensions of parental interaction may show substantial genetic effects, even though they do not have DNA. This

state of affairs is commonly referred to as the "nature of nurture" or genotype-environment correlation (see below for a more detailed description). It can be summarised as the degree to which "genetic factors play a role in individuals actively creating their own experience" (Plomin et al 1994). The nature of nurture has been examined for a range of measures of the family environment. More than half a dozen twin and adoption studies reported definite, though moderate, genetic effects on children's perceptions of parenting (Plomin 1994). Interestingly, these effects were higher for dimensions of warmth and negativity than for control. Other research, which will be discussed in more detail later on in this chapter, has also focussed on possible genetic influences on extra-familial environmental measures, such as life events, exposure to drugs, and complications of birth and pregnancy.

The above findings are of interest to psychiatry. Mental health professionals often work with clients' perceptions of aspects of their family environment, and may find knowledge of this nature-nurture confound useful. Also of interest are possible mediators of the genetic influence on environmental measures: since environments do not possess DNA, genetic effects on environmental measures must be mediated by a third variable. Saudino and Plomin argued that personality is a good candidate for mediation of genetic contribution to environmental measures since "genetically influenced personality traits could affect how people construct or perceive their environments" (Saudino et al 1996). Earlier, we provided a hypothetical example of such mediation involving extraversion as a mediator of genetic influence on experience of parental control. There has not been much research on personality as a mediator of genetic influences on the environment, but there are some suggestive findings. For example, in a project of the Swedish Adoption/Twin Study of Aging (which involved 320 MZ and DZ twin pairs reared together and reared apart) significant genetic effects were apparent for controllable life events (such as getting married or divorced), but not for uncontrollable life events (such as serious illness in a child or death of a spouse). In this sample, genetic effects of controllable life events was mediated entirely by measures of personality such as neuroticism, extraversion and openness to experience (Saudino et al 1997). Similar findings were reported in studies examining genetic influence on other environmental measures, although the mediating effect of personality for these measures was much weaker (Chipuer et al 1993; McGuire et al 1992).

3.3 Genotype-phenotype relationships

Most of our knowledge on the genetics of psychiatric diseases comes from the indirect approach of the study of psychiatric phenotypes in groups of related individuals. The main challenge to the indirect measurement of gene effects is that there is no straightforward,

one-to-one relationship between genotype and phenotype for many diseases, especially "complex" multifactorial diseases such as psychiatric disorders (Wolf 1995). For example, epidemiological models for multifactorial disorders predict that genes may be neither necessary nor sufficient to bring about disease (Susser 1991). This is referred to, in genetic terms, as incomplete *penetrance* (only a proportion of persons with the genotype will develop the disease), or *variable expressivity* if the outcome is something measured on a dimensional scale (such as neuroticism on the Eysenck Personality Inventory). Thus, absence of the phenotype does not mean absence of the genotype (false negatives). This can be compared to the concept of "carrier status" in infectious epidemiology (eg hepatitis B). For example, individuals who are heterozygous for the apolipoprotein E (ApoE) $\epsilon 4$ allele on chromosome 19 have a 3-fold elevated risk of developing Alzheimer disease (AD) (Corder et al 1993), but the majority of individuals with the ApoE- $\epsilon 4$ allele (around 13% in Caucasian populations) will never develop the disease (Henderson et al 1995).

The penetrance of a particular condition may be associated with exposure to environmental factors. For example, one study reported that the risk-increasing effect of the ApoE- $\epsilon 4$ allele on AD was modified by additional exposure to head injury. The authors studied the combined effect of head injury and genetic susceptibility on risk for Alzheimer's diseases (AD), using ApoE genotypes as a measure of genetic susceptibility. Classifying individuals with neither the ApoE- $\epsilon 4$ allele nor head injury as the reference group, they found that risk for AD was increased two-fold in individuals with ApoE- $\epsilon 4$ without head injury, and 10-fold in individuals with ApoE- $\epsilon 4$ and head injury (Mayeux et al 1995). These were recently replicated in another study (Teasdale et al 1997). Thus, penetrance appeared to be influenced by concurrent exposure to an environmental factor. The modification of genetic effects by environmental factors (or the other way round) is referred to as genotype-environment interaction and will be considered in more detail below with regard to functional psychiatric disorders.

Genetic penetrance may also be modified by other genes. This is referred to as gene-gene interaction or *epistasis*. For example, in some families where an Alzheimer's mutation at the APP (amyloid precursor) locus is transmitted, additional presence of ApoE- $\epsilon 4$ reduces the age-of-onset of disease in the individuals with the APP mutation (St George-Hyslop et al 1994). Epistasis is difficult to demonstrate, and usually is not considered in classical analyses of possible modes of transmission of psychiatric disorders. Risch, however, developed a method of analysing possible epistasis in aggregated data from family studies on schizophrenia, including first, second and third degree relatives. His results suggested the involvement of multiple, interacting genes at the exclusion of monogenic (ie involving a single gene) transmission (Risch 1990).

In some cases, a phenotype may be present without the associated genotype. This would be an example of an (environmental) *phenocopy*. For example, some forms of epilepsy have a minimal genetic contribution (ie are phenocopies), whereas a shared genetic susceptibility is suggested for others (Ottman et al 1996). Although similar models of subdivision of genetic and environmental cases have been proposed for psychiatric disorders (Murray et al 1985), there is little research to support their validity (Roy et al 1994). Thus, it is unlikely that most cases of schizophrenia are either due to genes *or* to environmental factors such as complications of birth and pregnancy, viral infections and cannabis.

A single gene often affects more than just a single phenotype, and may thus have two or more apparently different effects. This phenomenon is called *pleiotropy*. For example, ApoEε 4 not only increases the risk for AD, but also for coronary heart disease (Payne et al 1992; Van Brokxmeer et al 1992). Pleiotropy is an important concept in psychiatric genetics because most genetic influences on behaviour are likely to be pleiotropic. In mice, for example, it has been demonstrated that the gene for albinism also contributes to the variance of measures of emotionality (DeFries et al 1970). Mouse models are important in psychiatric genetics, because mouse and human DNA is very similar and behavioural traits in animals can be considered as crude analogues of human temperamental characteristics. Thus, the recent identification of three loci that explained all the variance of a behavioural dimension in mice that can be considered analogous to anxiety/depression in humans, has led to considerable interest in the corresponding human chromosomal regions as the site for loci that contribute to the variance of anxiety/depression in humans (Flint et al 1995). Pleiotropy may play an important role in psychiatry. For example, just as the pleiotropic effects of the gene causing phenylketonuria include mental retardation on the one hand, and lighter hair and skin color on the other, the pleiotropic effects of the genes contributing to the schizophrenia phenotype may include psychosis on the one hand, and, for example abnormalities in smooth-pursuit eye movements on the other.

Pleiotropy may also be relevant with regard to *co-morbidity*: one possible explanation for the fact that certain disorders occur more commonly together than would be expected by chance alone (Kessler et al 1994), is the pleiotropic effect of genetic factors. In an Australian study on 3198 twins pairs, the authors extracted a genetic factor which loaded onto both depression and anxiety, and which in both sexes accounted for around 30% of the total phenotypic variance. Other genetic and environmental factors that were extracted were smaller and tended to load differentially onto either anxiety or depression (Kendler et al 1987). These results therefore suggest a common set of genetic factors (but not a common set of environmental factors) for depression and anxiety. These findings were replicated in a study of over 1000 female twin pairs in Virginia, who, contrary to the great majority of twin studies, were ascertained from the general population and not from treatment facilities. This study found that the genetic components for anxiety and

depression were completely correlated, accounting for around 30% of the variance in liability to both disorders, whereas the environmental components were only partly correlated (Kendler et al 1992b).

The opposite of pleiotropy (one cause, multiple effects) is the situation where a single phenotype can be the result of more than one genetic influence. This phenomenon is referred to as *genetic heterogeneity* (one syndrome, multiple causes). For example, molecular genetic studies have demonstrated that mutations in three different genes are each sufficient to cause early-onset familial Alzheimer's disease (FAD), a rare form characterized by onset before 60 years of age. This disease has been linked to the amyloid precursor gene on chromosome 21 (Kamino et al 1992), the presenilin I gene on chromosome 14 (Schellenberg et al 1993), and the presenilin gene on chromosome 1 (Levy-Lahad et al 1995). Although genetic heterogeneity has thus been shown to play an important role in FAD, it is probably less important in "functional" psychiatric disorders such as schizophrenia and affective disorder. This is because it is very unlikely that psychiatric disorders, unlike FAD, are caused by single sufficient genes (McGue et al 1989). The sort of heterogeneity one is likely to encounter in the molecular genetics of psychiatric disorders is one where only a minority of cases involving rare pedigrees are caused by a gene of major effect, whereas the majority of cases involve multiple genes of small effect that in interaction with each other and environmental factors contribute to the underlying disease liability.

3.4 Heritability

Heritability is a concept that often gives rise to confusion. It can be described as the degree to which genetic *differences* among individuals in a certain population make a *difference* at the level of the phenotype. The concept of heritability is purely descriptive, and *not* predictive, as is often assumed. For example, twin studies have demonstrated that concordance rates for schizophrenia are higher in genetically identical monozygotic twins (who share 100% of their genes) than in dizygotic twins (who share on average only 50% of their genes). Greater MZ than DZ similarity on a trait suggests a genetic influence, because MZ twins are more similar genetically than DZ twins. Using twin data based on comparisons of large numbers of MZ and DZ pairs, it has been estimated that the *heritability* of schizophrenia is around 70%, meaning that around 70% of the differences in *liability* to schizophrenia is due to differences in genetic make-up (Kendler 1983). However, the real figure may be lower than 70%, because the twin data on which heritability estimates are based assume that monozygotic and dizygotic twins are equally similar with regard to environmental risk factors, whilst the available evidence suggests

that this is not the case with respect to, for example, the early intrauterine environment (Phillips 1993). Thus, the higher concordance rates for schizophrenia in MZ twins as compared to DZ twins may be in part related to the fact that MZ twins more often share the same early environmental risk factors (Davis et al 1995). Heritability estimates may be further inflated because of failure to take into account interactions between genes, and interactions between environmental and genetic factors in the causation of schizophrenia (Gottesman et al 1983). If there is genetic control of sensitivity for environmental factors (see below), subsequent environmental changes can have a dramatic impact on the heritability of a disorder (Vogel et al 1986). It is also necessary to be aware of the fact that heritability depends on the population prevalence of a disorder. If disorders A and B have similar differences in concordance rates between monozygotic and dizygotic twins, disorder A will have a higher heritability estimate if its population prevalence exceeds that of disorder B (LaBuda et al 1993).

Most measures of psychopathology have been subject to genetic-epidemiologic investigations to estimate their heritabilities. The range of values of MZ and DZ concordance rates (from which heritability is estimated) for different measures of psychopathology have been summarised in figure 2, based on major reviews and studies (Begleiter et al 1995; Craddock et al 1995; Kendler 1983; Kendler et al 1992a; Kendler et al 1992c; Loehlin 1992; McGuffin et al 1984a; McGuffin et al 1991; McGuffin et al 1994; McGuffin et al 1993). It is clear that all major psychiatric phenotypes show some genetic influence, and that this influence is more important for affective disorders and schizophrenia than for phobias and alcoholism. Heritability estimates have also been calculated for normal personality traits, which is relevant because some personality traits are risk factors for psychiatric disorders. It has been established, for example, that nearly 50% of differences in neuroticism, extraversion and general intelligence can be attributed to genetic differences (Plomin et al 1994).

Fig. 2 Concordance rates for psychiatric disorders in monozygotic and dizygotic twins

Heritability does not imply stability. Even for personality dimensions which have some degree of age-to-age stability, as is the case for most (Costa et al 1994), different genes can be involved at different stages of development, and different influences of genes and environment may also be present at different ages. For example, juvenile delinquency has a strong familial component, which does not seem to be due so much to genetic influences, but to the influence of the shared familial environment. Contrary to juvenile delinquency, however, twin studies of adult criminality do suggest a definitive genetic component (Lyons et al 1995; McGuffin et al 1984b).

3.5 How many genes?

The marked fall-off in liability with genetic distance from monozygotic twin to second-degree relative (Fig. 3) suggests that liability to schizophrenia is not influenced by a single gene, because the probability that a combination of alleles is transmitted together decreases much more rapidly with genetic distance than transmission of a single allele (Sham 1996). Thus, a "multilocus genetics of schizophrenia" (Cloninger 1997) may be the most appropriate model, each of the genes carrying a small effect, possibly interacting with each other, and none of the genes in itself being either necessary or sufficient to bring about illness (Benjamin et al 1996). The rapid fall-off in risk with genetic distance is not specific to schizophrenia. A similarly rapid fall-off is observed in affective illness such as bipolar disorder (morbid risk MZ co-twin of affected individual: 60%; morbid risk sib of affected individual: 0.07; no reliable data on second degree relatives are available (Craddock et al 1995)).

Fig. 3 Multiple genes for schizophrenia?

3.6 Which phenotype?

It has been pointed out that multiple-gene influences on complex characteristics result in continuous dimensions rather than dichotomous disorders (Plomin et al 1994). Recent work

has suggested that psychotic disorders may be best represented by several symptom dimensions (Van Os et al 1996b), that may be part of a psychosis continuum (Taylor et al 1993). Taylor proposed a genetic basis for a continuum of affective and non-affective psychosis (Taylor 1992; Taylor et al 1993). These views received strong support from the Roscommon Family Study, which was one of the very few family studies to use an epidemiologic approach to proband sampling. The authors of this study reported that "the familial liability to schizophrenia is, at least in part, a liability to develop psychosis" (Kendler et al 1993a). Similarly, a twin study by Farmer and colleagues found that the most "genetic" definition of schizophrenia (ie the diagnostic definition giving the highest difference between MZ and DZ concordance rates) was the one that included affective psychosis and atypical psychosis (Farmer et al 1987). Within the functional psychoses, certain symptom dimensions predict high familial morbid risk of psychosis (Cardno et al 1997; Farmer et al 1984; McGuffin et al 1984a; Van Os et al 1996b), and may be suitable for molecular genetic approaches to detect genes of small effect contributing to continuously distributed characteristics. These developments are interesting, because there is also renewed interest in the possible links between personality and psychopathology. The dimensional representation of psychopathology facilitates conceptually the view that some psychiatric disorders may be seen as the extreme manifestations of the normal distribution of personality traits. From the perspective of genetic epidemiology, if psychopathology is considered an extreme manifestation of normal personality, then the genes contributing to personality should also contribute to psychopathology. In the earlier mentioned Roscommon Family Study, the risk of schizophrenia in the relatives of schizophrenic probands was not different from that found in the relatives of probands with schizotypal personality disorder, indicating that schizophrenia shares a familial predisposition with schizotypal personality disorder (Kendler et al 1993a). Interestingly, in the siblings of probands with schizophrenia, a parental diagnosis of schizotypal personality disorder increased the risk for schizophrenia fourfold (Kendler et al 1995b). In a study of 1733 female twins from a population-based twin registry in Virginia, Kendler and colleagues found that around 55% of the genetic liability of major depression was shared with neuroticism (Kendler et al 1993c). Similar results had been reported in earlier study by Eaves and colleagues (Eaves et al 1989). Thus, although few studies have addressed the question, the available evidence suggests genetic overlap between personality and some areas of psychopathology. Better methods to examine whether facets of psychopathology are genetic extremes of personality are being developed (Plomin et al 1991).

The possible links between normal personality and psychopathology are also of interest to clinicians because they require a reconceptualization of what is psychological dysfunction and what is required as a therapeutic approach. For example, phenomena such as delusions and hallucinations are quite common in the general population (Eaton et al 1991; van Os et

al 1999; Verdoux et al 1998), and it has been pointed out that such experiences *themselves* may actually be neutral with respect to psychopathology. However, the way they are *reacted to* by individuals may actually generate psychological distress which subsequently is labeled as psychopathology (Claridge 1997).

4. Genotype environment interaction

Relatively low schizophrenia concordance rates (around 50%) in pairs of genetically identical MZ twins suggest that environmental factors play an important and independent role in the pathogenesis, either by causing "phenocopies" (non-genetic cases), or by influencing expression of disease in genetically vulnerable individuals. Two studies examined the risk of schizophrenia in the offspring of affected and non-affected co-twins of discordant MZ pairs, and found that the risk was elevated in not only the children of the affected co-twins, but also in the children of the well co-twins (Gottesman et al 1989; Kringlen et al 1989). These findings not only suggest that schizophrenia phenocopies are rare, but also that environmental factors may be necessary for the disease to become manifest in individuals with predisposing genotypes. Similar results were reported for bipolar disorder in another twin study examining the offspring of normal and affected co-twins (Bertelsen et al 1986). In other words, the effect of genes appears to be probabilistic rather than deterministic, and psychiatric disorders such as schizophrenia and bipolar disorder may be the result of a mechanism of genotype-environment interaction.

Genotype-environment interaction refers to a genetically mediated sensitivity to environmental factors (Kendler et al 1986). Thus, some genotypes are more likely than others to develop disease in the event of exposure to certain environmental factors (Fig. 4). In the case of genotype-environment interaction, diseases will tend to cluster in families not because of a direct genetic effect, but because relatives are more vulnerable to the risk-increasing effect of a prevalent environmental risk factor. It is becoming increasingly clear that genotype-environment interaction plays a role in many multifactorial diseases. For example, researchers are in the process of identifying several asthma susceptibility genes, that mediate allergic responses to various environmental factors (Casolaro et al 1996; Shirakawa et al 1994). Similarly, it has been proposed that the mechanism underlying diabetes is a genetically mediated sensitivity to viruses or dietary factors (Graves et al 1997; Perez-Bravo et al 1996; Pettitt et al 1997), and other research evidence indicates that genetically mediated sensitivity to nutrients and smoking are playing a role in cardiovascular disease (Breslow 1996) and COPD (Khoury et al 1986) respectively.

Fig. 4 *Gene-environment interaction: genetic control of sensitivity to the environment*

A special type of genotype-environment interaction, the so-called stress-vulnerability model, is generally believed to reflect disease aetiology in psychiatric disorders such as psychosis and depression (Zubin et al 1977). According to this model, individuals differ in their sensitivity to adverse environmental circumstances, and genetically sensitive individuals are more likely to develop symptoms when exposed to these environments than others. Until recently, however, very little evidence was provided to support this thesis. The Finnish Adoptive Family Study of Schizophrenia followed the adopted away offspring of mothers with schizophrenia, and compared them with control adoptees for frequency of schizophrenia spectrum outcomes. It was shown that the risk of developing schizophrenia spectrum disorders in high-risk adoptees was greater than for control adoptees, but only for those high-risk adoptees who were additionally exposed to a dysfunctional family rearing environment (Tienari et al 1994). Interestingly, high-risk individuals were not only more at risk of developing schizophrenia spectrum disorders when exposed to additional environmental adversity: A subsequent investigation by the same group (Wahlberg et al 1997) showed that the presence of schizophrenia in the biological mother together with the presence of communication deviance in the adoptive parents also carried the highest risk of developing schizophrenia *vulnerability*, as measured by the Rorschach Index of Primitive Thought. Thus, the Finnish studies suggest that genetic liability to schizophrenia is only expressed in the presence of additional adverse environmental factors.

Cumulative exposure to life events increases the risk for schizophrenia (Bebbington et al 1993). A recent study suggested that the experience of life events in psychotic patients is not independent of genetic diathesis: the risk for schizophrenia in the first degree relatives of patients whose psychotic episode was preceded by a stressful life event was higher than the morbid risk for schizophrenia in the first degree relatives of patients without exposure to life events (Van Os et al 1994). Although open to more than one explanation, these

results are compatible with a genetically mediated sensitivity for stress in schizophrenia. Depression resembles schizophrenia in that it is also characterized by neurodevelopmental impairment (van Os et al 1997b) and cerebral ventricle enlargement (Elkis et al 1995), is also extremely reactive to life events and difficulties, and shows familial-genetic overlap with schizophrenia (Van Os et al 1997). A recent study of over 1000 female-female monozygotic twins reported that the risk for onset of depression following a life event was around twice as high for the twins who were also at the highest genetic risk for depression (Kendler et al 1995a). These results therefore suggest that genetic control of sensitivity to social precipitants plays a role in the causation of depression. Another type of genotype-environment interaction was demonstrated in an adoption study by Cadoret and colleagues (Cadoret et al 1996). The authors studied the concept of depression spectrum disease, a type of major depression characterized by families in which male relatives are alcoholic and females are depressed. Onset of depression in the 197 adopted away offspring of alcoholic biological parents was examined in relation to additional exposure to environmental adversity. In female adoptees, major depression was predicted by an alcoholic diathesis only in the presence of additional environmental adversity, such as psychological disturbance in the adoptive parent, or psychiatric problems in the adopted sibling. No such genotype-environment interaction was found in male adoptees.

Genetic factors may influence sensitivity to environmental risk factors for psychiatric disorder also in an indirect way. For example, the impact of stressful life events may be mediated by the individual's capacity to solve problems, seek support and otherwise cope with adversity. If such coping mechanisms themselves are under genetic influence, the buffering effect of coping mechanisms on the impact of stressful life events would constitute another example of genotype-environment interaction. Support for such mechanism comes from a large twin study, in which it was estimated that the heritability of coping mechanisms was around 30% (Kendler et al 1991).

Walker and colleagues followed two groups of high risk males (offspring of schizophrenic mothers). The first group had displayed patterns of electrodermal lability in childhood and later developed schizophrenia, and the second group had also displayed electrodermal lability but did not subsequently develop the disease. The schizophrenic group had experienced significantly more early environmental adversity than the non-schizophrenic group, characterised by paternal absence during early life (Walker et al 1981). While it is known that loss of the father early in life in itself does not increase the risk for later schizophrenia (Huttunen et al 1973), the results of this study suggest that early loss of the father *in combination with* genetic vulnerability can.

An interesting type of genotype-environment interaction in schizophrenia was proposed by Wright and colleagues (Wright et al 1993a). These authors suggested that schizophrenia is the result of an autoimmune disorder of the mother-foetus combination. A genetically mediated maternal immune response to an environmental trigger such as influenza, could interfere with foetal neurodevelopment if, for example, the maternal antibodies cross-reacted with proteins in the developing foetal brain. This theory has the advantage of bringing together neuropathological, epidemiological and immunological findings in schizophrenia and of being easily falsifiable. Consistent evidence, however, has as yet not been produced, although some tentative findings were reported by the same group (Wright et al 1993b).

In bilaterally symmetrical organisms, including man, paired structures develop as mirror images of each other. Random deviations from bilateral symmetry are called fluctuating asymmetry (FAS). The ridge counts of the hands and fingers are examples of such bilateral traits which are often used in the study of FAS. Ridge counts have high heritabilities and are under polygenic control, but they are also subject to adverse intra-uterine influences such as viral infections and exposure to alcohol (Fananas et al 1996; Schaumann et al 1976). FAS occurs when, during the development of an organism, environmental factors interfere with the ability of that organism to execute its developmental program the same way in both sides (Newell-Morris et al 1989). Some individuals are better able than others to buffer these environmental interferences. There is evidence to suggest that this buffering ability is dependent on the individual's genotype, in particular the relative number of heterozygous loci (less heterozygosity being associated with less buffering ability and more FAS) (Clarke et al 1986; Livshits et al 1991). For psychiatric disorders such as schizophrenia, this concept of genetically determined ability to ward off developmental stressors is of interest and can be tested in samples of monozygotic twins with the disorder. Markow and Gottesman examined a sample of 22 (identical and fraternal) twins concordant and 19 pairs discordant for schizophrenia. They found that twins concordant for schizophrenia had higher levels of ridge count FAS than discordant pairs (Markow et al 1989). These findings are open to several explanations, one of which is that concordant twins are more vulnerable for environmental adversity through a genetically determined reduced ability against environmental stressors. Support for this hypothesis came from another twin study, where 24 MZ twins concordant and discordant for schizophrenia were compared with 13 control pairs. It was found that MZ twins concordant for schizophrenia were around 6 times more likely than control twins to carry dermatoglyphic abnormalities associated with intra-uterine environmental stressors, whereas no such differences were apparent in the comparison with the discordant twins (van Os et al 1997a).

A somewhat paradoxical feature of genotype-environment interaction is that the heritability estimates of a disorder go up in predisposing environments (Kendler 1995). This principle

can be illustrated with phenylketonuria (PKU), a paradigm example of a disease resulting from genotype-environment interaction. In environments where there is no phenylalanine, the disease cannot develop even in the presence of the disease gene. The disease will therefore not cluster in families even though the susceptibility gene is transmitted; heritability is zero. In environments where exposure to phenylalanine is very common, however, disease rates will be high and familial clustering will be obvious. Thus, PKU heritability will go up in predisposing environments. Several studies have now suggested that a similar mechanism may be operating in schizophrenia. For example, it has been established that incidence rates of psychosis in certain ethnic minority groups, such as UK and Dutch African-Caribbean groups, are substantially elevated (Selten et al 1997; Van Os et al 1996a; van Os et al 1996). Two studies have now shown that the rates of psychosis in the siblings of African-Caribbean probands are *also* elevated as compared to the siblings of white probands (Hutchinson et al 1996; Sugarman et al 1994). These elevated familial rates suggest that a mechanism of genotype-environment interaction may be operating. The explanation for the differential familial morbid risks in African-Caribbean and white probands may be excess exposure to a predisposing environment in ethnic minority groups, such as racial life events, discrimination and the stresses of acculturalisation (McKenzie et al 1995).

Genes may interact not only with adverse rearing environments and psychosocial stressors, but also with early risk factors such as complications of birth and pregnancy (PBC's). In one study, three categories of genetic exposure were defined on the basis of the presence of schizophrenia and related disorders in neither, one or two parents. Ventricular enlargement was examined in these three groups in relation to PBC's. It was shown that the association between PBC's and ventricular enlargement was stronger in individuals at high genetic risk than in individuals at low genetic risk (Cannon et al 1993). Parnas and colleagues reported on a follow-up of 207 children from mothers with severe schizophrenic illness. The risk of developing schizophrenia in this high-risk sample was highest for those who had additional exposure to complications of birth and pregnancy, derived from standardized birth records (Parnas et al 1982). These findings, like those from the earlier cited studies, again suggest that vulnerability to schizophrenia is only expressed in the presence of additional adverse environmental factors.

Thus, several studies have suggested that genotype-environment interactions play a role in at least a proportion of schizophrenia cases. The measures used, however, were crude and the length of follow-up of high risk samples limited. The results nevertheless suggest that genotype-environment interaction constitutes a mechanism in the causation of psychosis. While we have focussed here mainly on psychosis and depression, similar findings have been reported for a range of psychiatric disorders, such as conduct disorder and autism

(Folstein et al 1977). Particularly important was an adoption study by Cadoret and colleagues, who showed that the adopted away children from parents with antisocial personality disorder had a high risk of developing antisocial behaviours themselves if they grew up in adverse adoptive home environments, characterised by, for example, adoptive parents with marital problems, substance abuse, legal problems and anxiety conditions. This study once again points to the important role of the environment in mediating genotype-phenotype relationships (Cadoret et al 1995).

5. Genotype environment correlation

Another type of genotype-environment relationship, which was already touched upon above, is one where genes do not (only) control sensitivity to an environmental factor, but (also) control *exposure* to an environmental factor (Fig 5). Thus, individuals may become more liable to disease because their genetic make-up predisposes them to select themselves into high-risk environments. In the case of genotype-environment correlation, diseases will tend to cluster in families not because of a direct genetic effect, but because relatives more often expose themselves to the environmental risk factors for disease. For example, individuals may not only have genetically determined differences in their *sensitivity* to alcohol (genotype-environment interaction), but also genetically determined differences in their propensity to *start using* alcohol in the first place (Begleiter et al 1995).

Fig. 5 Gene-environment correlation: genetic control of exposure to the environment

Use of cannabis is a risk factor for schizophrenia (Andreasson et al 1987). Not only are there genetically determined differences in the individual psychotropic effects produced by cannabis (Lyons et al 1997), but use of cannabis itself is also influenced by genetic factors (Tsuang et al 1996). Thus, part of the genetic contribution to schizophrenia may be mediated by the use of cannabis and its subjective effects (genotype environment

correlation and interaction). McGuire and colleagues found that the risk for schizophrenia in the first degree relatives of schizophrenic patients who screened positive for cannabis was increased more than tenfold compared to schizophrenic patients who screened negative (McGuire et al 1995). These results are suggestive of a mechanism of genotype-environment interaction and/or genotype-environment correlation, as in both instances the first degree relatives of cases exposed to the environmental risk factor should have higher rates of illness (Marcelis et al 1998).

A number of studies have now also suggested that the occurrence of life events is not random. For example, the heritability estimates for wartime exposure to traumatic events was found to be between 35% and 47% in a recent study of 4,029 male-male twin pairs who served in the United States military during the Vietnam war (Lyons et al 1993). Other studies examining the types of life events that are known to contribute to the onset of psychosis and depression have also suggested that part of the reason for familial aggregation of psychiatric disorders such as depression is due to the tendency of biological relatives to more often expose themselves to high-risk stressful life events (Kendler et al 1993b; McGuffin et al 1988). One recent study estimated that around 10-15% of the impact of genes on risk for major depression is mediated through stressful life events (Kendler et al 1997).

6. Genotype environment interaction and molecular genetics

Although there are as yet no definitive results, it is likely that susceptibility genes (ie genes that increase the risk for psychiatric disorder but are neither necessary nor sufficient in themselves to cause illness) for psychiatric disorder and normal personality traits will be identified in the foreseeable future (Detera-Wadleigh et al 1996; Lesch et al 1996; Peltonen 1995; Straub et al 1994; Williams et al 1996). The discovery of such genes may paradoxically greatly enhance our capacity to use environmental manipulations to prevent and cure disease in the presence of genotype-environment interaction or genotype-environment correlation. For example, it will be possible to classify individuals according to the high-risk genotype with a much greater degree of precision than was hitherto possible, and to investigate directly which genotypes mediate sensitivity to, or exposure to, a particular predisposing environment. For example, genetic polymorphisms in some individuals with schizophrenia may be linked to exposures such as cannabis, that set of their psychotic episodes. In the future, similar investigations may be possible in psychiatric disorders such as schizophrenia and affective disorder, maybe allowing for specific environmental counselling (rather than genetic counselling) on the basis of the presence of specific susceptibility genes (Editorial 1996b).

It has also been pointed out that, with the advent of molecular genetics, it may be possible to improve the predictive value of risk factors. For example, a number of publications about detection of schizophrenia in the *prodromal* phases has recently seen the light (McGorry et al 1996; Yung et al 1996), but it has been argued on epidemiological grounds that the predictive values of all the known risk factors for schizophrenia, including the presence of a first degree relative with schizophrenia, are too low to be of use in clinical practice (Jones et al 1997; van Os et al 1997c). It has been shown, however, that if the risk-increasing effect of an environmental risk factor for schizophrenia is confined to a particular genotype (that is, if there is genotype-environment interaction), the predictive value of the risk factor may be increased up to 50-fold if individuals can be classified according to the high-risk genotype (Khoury et al 1995).

7 Conclusion

The emerging field of genetic epidemiology appears to have both theoretical, clinical and public health importance with regard to psychiatric disorders, especially as regards i) the role of the environment in bringing about psychiatric disease and shaping personality development, ii) the study of co-morbidity of psychiatric disorders, and iii) genotype-environment interactions in the aetiology of psychiatric illness. Developments in molecular genetics need to go hand in hand with progress in the study of environmental factors that interact with genes; for the time being it is easier (and ethically preferable) to change the environment than it is to change one's genes, and even if the latter possibility existed, it could not be justified ethically as an intervention at the level of public health. Prevention of genotypes as a public health measure can never be acceptable in psychiatry (Khoury 1997). However, prevention of phenotypes by targetting environmental risk factors that interact with genotypes is. Mental health professionals, as experts in the area of environmental adversity in relation to psychiatric disorder, should take the lead in making molecular genetic findings relevant to clinical practice.

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CHAPTER 2

Clinical studies of this thesis – outline and aims

Research strategy to identify environmental risk factors

Investigating the differential contribution of genes and environment and the way they might interact/correlate is fraught with difficulties, as explained in chapter one. If the hypothesis of gene-environment interactions contributing to schizophrenia risk is valid, then it should be possible to demonstrate effects of selected environmental risk factors. This, however, is no easy task as genes themselves may contribute to the likelihood of environmental risk exposure. Special study designs may be invoked to investigate the possible contribution of environmental factors to the causation of schizophrenia. Incidence studies of schizophrenia using different exposure variables have been helpful in this respect. For example, differences in the incidence of schizophrenia have been found to be associated with time period, extreme exposures such as nutritional depletion during war time, geographic area, migration status, gender and social class (see table 1). Variations in incidence figures associated with these variables, especially geographical and temporal variation, are suggestive for the presence of environmental factors influencing the risk for schizophrenia. This thesis starts with a study examining one of these presumed environmental factors that may play a role in the causation of schizophrenia, ie. the degree of urbanization of place of birth.

The 'urban factor' as an environmental risk factor in schizophrenia

In *Chapter 3* of this thesis we investigated by means of a retrospective follow-up of a birth cohort whether the degree of urbanization of place of birth was associated with the cumulative risk for schizophrenia. It was examined which individuals are most at risk and whether the risk-increasing effect is specifically related to schizophrenia or whether it can also be found in other diagnostic categories. We attempted to determine how plausible it is that this 'urban factor' is a proxy measure for an environmental risk factor, because there

are several suggestions that the increased incidence rates in urbanized areas are due to immigration of genetically predisposed individuals.

Table 1. *Variation in incidence as a clue to environmental causes of schizophrenia*

	<i>Indicating presence of:</i>	
	<i>Genetic factors</i>	<i>Environmental factors</i>
•Temporal variation	-	+
•Extreme exposures	-	+
•Spatial variation	(+)	+
•Migrant variation	-	+
•Gender differences	+	+
•Class variation	-	+

Urban birth or urban residence?

Place of birth and place of later residence are probably highly correlated. However, none of the studies to date investigating urbanization in relation to schizophrenia, have been able to differentiate between an effect of urbanization of place of birth or that of later residence. In *Chapter 4* we further investigated the 'urban factor' in order to determine *when* the exposure of the presumed environmental risk factor for schizophrenia has its effect. Is it a factor that operates early in life (before onset of disorder) or is it more likely to operate around the time of illness onset? Clarifying the time window during which the exposure takes places may help to elucidate the specific risk factor(s) associated with urbanization that may contribute to the liability for schizophrenia.

Research strategy to identify genotype-environment correlation

There are several, increasingly sophisticated, techniques to investigate genotype-environment correlation in the case of a trait for which the liability genes have not yet been identified. These are dependent on the type of genotype-environment correlation under examination (passive, evocative, or active genotype-environment correlation (Plomin et al 1977)). Passive genotype-environment correlation can be detected by comparing correlations between family environment and children's traits in nonadoptive and adoptive families. Evocative and active genotype-environment correlation can be detected by the correlation between the biological parents' traits (as an index of adopted children's genotype) and the environment of adoptive families. A third method to detect genotype-

environment correlation involves multivariate genetic analysis of the correlation between an environmental measure and a trait. This can be used for detection of all three types of genotype-environment correlation. The multivariate genetic analysis estimates the extent to which genetic effects on one measure overlap with genetic effects on another measure. Genotype-environment correlation is implied if genetic effects on an environmental measure overlap with genetic effects on a trait measure (cross-twin-cross-variables design). Thus, an environmental measure in one twin can be correlated with a trait measure in the other twin. The degree of genetic mediation of this phenotypic correlation can be assessed by comparing these sibling cross-correlations between nonadoptive and adoptive siblings. If the correlation in non-adoptive siblings is higher than that in adoptive siblings, this indicates genetic mediation. Because of the low incidence of schizophrenia it is very difficult to investigate large samples of subjects (especially twins), and small sample sizes do not have enough power to detect differences. We have, therefore, developed another method to investigate gene-environment correlation.

Obstetric complications in relation to familial morbid risk of psychiatric disorder as a paradigm to elucidate genotype-environment correlation

Research suggest that so-called "obstetric complications" (hereafter: OCs) occur more frequently in patients with schizophrenia than in normal controls (Geddes et al 1999; Verdoux et al 1997). There is still debate about the nature of this phenomenon. Several interpretations may be appropriate, one of which is that the genes that contribute to schizophrenia liability also influence the likelihood to experience OCs which would be compatible with a model of genotype-environment correlation. In *Chapter 5* of this thesis, we tested this hypothesis by examining whether the morbid risk of psychotic illness is higher in first-degree relatives of both schizophrenia and control probands exposed to obstetric complications (OCs), compared to probands not exposed to OCs.

Research strategy to identify genotype-environment interaction

A method that has been frequently used to investigate genotype-environment interaction is to correlate a positive family history for schizophrenia with certain proxy environmental variables, such as ethnic group, urban birth, obstetric complications, winter/spring birth, stressful life events. This method, however, is not very accurate because of important limitations:

1. Results are difficult to interpret unless both the environmental exposure status and the clinical outcome status are measured in both cases and all first degree relatives and analyses are adjusted for age, sex and number of relatives.

2. There will be a high level of misclassification as many unaffected relatives may carry the high-risk genotype
3. It is not informative because absence of an association between positive family history and environmental exposure does not rule out gene-environment interaction, and presence of an association does not rule out lack of gene-environment interaction (see Chapter 5).

Specific twin, adoption and high-risk study designs are more informative with regard to the study of genotype-environment interaction. In addition, structural equation modelling can be helpful to detect genotype-environment interaction in twin studies. Even though twin- and adoption studies have shown to be indicative of differential environmental effects as a function of genotype (Tienari et al 1994; Wahlberg et al 1997), the problem with all these studies remains the control over the environmental exposure. It often remains difficult to assess what is actually measured: genetic effects or environmental effects. In animal studies, it is easier to detect genotype-environment interaction than in clinical studies on human disease outcomes. This is because the environmental exposure can be manipulated and controlled in laboratory conditions. In human research, such as life event research, control of the environmental exposure is not possible and therefore subject to uncontrolled genetic effects as explained in Chapter 1. This thesis describes another method to detect genotype-environment interaction in studies of human psychopathology that circumvents the above described problem of an uncontrollable environment, by *creating* a well controlled, standardized environmental exposure, eg. metabolic stress. Metabolic stress can be induced in humans by the infusion of the glucose-analog 2-deoxy-glucose (2-DG) that competes with normal glucose in the human body, according to the paradigm developed by Breier and colleagues (Breier et al 1993). The effect of this metabolic stressor on plasma homovanillic acid (HVA), a metabolite of central and peripheral dopamine and noradrenaline, and cortisol can be measured in plasma. Plasma HVA does not purely reflect central nervous system dopamine (DA) activity, as it is largely derived from peripheral catecholamine activity (Amin et al 1992; Kopin et al 1988a; Kopin et al 1988b). There is substantial evidence, however, that suggests that plasma HVA does reflect to a degree the amount of central DA activity and indeed can be used as a marker of central DA activity (Friedhoff et al 1997). More important with regard to our stress paradigm, however, is the fact that the general stress reaction in the body, accompanied by elevated cortisol and HVA levels, is nevertheless eventually under the control by central regulatory factors, and thus associated with higher brain function (Breier et al 1993).

Metabolic stress as a paradigm to elucidate genotype environment interaction

Prior research has shown that patients with a diagnosis of schizophrenia have a stronger increase in HVA levels as compared to healthy controls during metabolic perturbation. Chapter 6 describes a family study in which a metabolic stress paradigm (Breier 1989;

Breier et al 1993; Breier et al 1988) is used to examine whether there is evidence for a model of genotype-environment interaction in schizophrenia. Patients with psychosis, non-psychotic relatives of patients with psychosis, and controls all underwent double blind infusion of 2-DG and placebo on two separate days. It was examined whether the effect of the metabolic stressor on plasma HVA and cortisol levels was mediated by the genetic liability for psychosis. The rationale for the inclusion of the first degree relatives is that these individuals are at a higher risk to develop schizophrenia (increase in risk approximately 10-fold), and it is generally accepted that this increase in risk, resulting in clustering of schizophrenia within families, is the result of shared genes rather than shared environmental factors. Therefore, being a first degree relative of a patient with schizophrenia can be considered as a proxy variable for genetic risk. The hypothesis of gene-environment interaction involving metabolic stress can then be tested by examining the pattern of HVA and cortisol response to the stressor in three groups: general population controls, first degree relatives of patients with psychosis and patients with psychosis. If there is no gene-environment interaction, the pattern of HVA and cortisol response would be like depicted in figure 1. In the case of gene-environment interaction, the pattern of response would be that the response of the relatives would be intermediate (or equal depending on the level of shared genes) to that of the patients (figure 2).

Research strategy to identify indicators of genetic risk (endophenotypes)

Structural cerebral endophenotypes

Various studies have shown that structural brain abnormalities cannot only be found in patients with schizophrenia but also in their healthy first-degree relatives, suggesting genetic influence. Although there are many inconsistencies in the neuroimaging literature, there is a large body of evidence derived from CT and MRI studies for ventricular enlargement (Johnstone et al 1976; Raz et al 1990), frontal lobe deficits (Andreasen et al 1994b; Breier et al 1992; Goldstein et al 1999), temporal lobe deficits with specific emphasis on the hippocampus/amygdala (Kelsoe et al 1988; Nelson et al 1998; Velakoulis

et al 1999), thalamic abnormalities (Andreasen et al 1994a; Buchsbaum et al 1996; Flaum et al 1995), as well as for cerebellar alterations (Katsetos et al 1997; Levitt et al 1999; Nopoulos et al 1999; Volz et al 2000). The traditional method to image analyses usually concerned manual tracing of *a priori* defined structures. *Chapter 7* describes a study in which structural brain differences between psychotic patients, non-psychotic first-degree relatives and healthy controls are investigated using a voxel-based approach to MRI data analyses which yields the advantage of examining the whole brain at once. Grey matter density differences were compared on a clustered-voxel wise basis. The aim of the study was to investigate whether there are brain structure abnormalities that may be markers of genetic transmission, again using relative status as a proxy measure of genetic risk.

Chapter 8 provides a summary of the main findings and a discussion of implications for clinical practice and future research.

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CHAPTER 3

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Urbanization and psychosis: a study of 1942–1978 birth cohorts in The Netherlands

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ABSTRACT

Background. Urban birth is associated with later schizophrenia. This study examined whether this finding is diagnosis-specific and which individuals are most at risk.

Methods. All live births recorded between 1942 and 1978 in any of the 646 Dutch municipalities were followed-up through the National Psychiatric Case Register for first psychiatric admission for psychosis between 1970 and 1992 ($N = 42115$).

Results. Urban birth was linearly associated with later schizophrenia (incidence rate ratio linear trend (IRR), 1.39; 95% confidence interval (95% CI), 1.36–1.42), affective psychosis (IRR, 1.18; 95% CI, 1.15–1.21) and other psychosis (IRR, 1.27; 95% CI, 1.24–1.30). Individuals born in the highest category of the three-level urban exposure were around twice as likely to develop schizophrenia. Associations were stronger for men and for individuals with early age of onset. The effect of urban birth was also stronger in the more recent birth cohorts.

Conclusions. There are quantitative differences between diagnostic categories in the strength of the association between urban birth and later psychiatric disorder. High rates of psychosis in urban areas may be the result of environmental factors associated with urbanization, the effect of which appears to be increasing over successive generations.

INTRODUCTION

Having been born or brought up in urban environments increases the risk of adult schizophrenia (reviewed by Freeman, 1994), suggesting that early environmental risk factors make individuals more susceptible to later illness, possibly by affecting early brain development (Torrey & Bowler, 1990; Lewis *et al.* 1992; Takei *et al.* 1992). In addition, the risk of urban birth appears to be potentiated by birth in winter months, a finding that has been interpreted as evidence that viral infections may be the risk-increasing agent (O'Callaghan *et al.* 1995; Takei *et al.* 1995a; Torrey *et al.* 1997).

In the present study, we wished not only to

examine the consistency of the above findings in a larger national sample, but also to address the following issues. First, it is not known whether the risk-increasing effect of an urban environment is specific to schizophrenia or whether, for example, the same applies to affective psychosis. Secondly, Eaton (1974) noted that there were differences between urban and rural environments in the age at onset distribution of schizophrenia. This may be an indication that the effect of urban environments is not the same for all demographic groups and needs further investigation. Thirdly, the decades following the Second World War have seen a great increase in the level of urbanization. In the Netherlands, for example, the population grew from 8.99 million in 1942 to 15.13 million in 1992, making the country one of the most densely populated areas in Europe. The three provinces of Noord-Holland, Zuid-Holland and Utrecht (the

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Randstad, population: 6.7 million) increasingly constitute a joint, densely populated megapolis. The increase in urbanization did not affect all regions similarly, resulting also in a continuing rise in the contrast between regions (Rijksinstituut voor Volksgezondheid en Milieu, 1997). Therefore, if urban birth and upbringing affect the risk of adult schizophrenia, one would expect a corresponding increase in its relative risk over time in the Netherlands. Finally, because a growing proportion of the world population is living in an urban environment, any risk-increasing effect is likely to have a large public health impact. This impact can be quantified in terms of the population attributable fraction, that is, the proportion of cases that could be prevented if the risk factor was removed (assuming that the risk factor exerted a causal influence).

METHOD

Birth cohorts

The Dutch governmental Central Bureau of Statistics (CBS) provided figures on all yearly live births by sex recorded between 1942 and 1978 in any of the 646 Dutch municipalities, and on all monthly live births by sex recorded in any of the 11 regions between 1953 and 1978 (no data on monthly live births by region were available for the years 1942–1952). These cohorts were followed-up through the National Psychiatric Case Register for first psychiatric admission for psychosis between 1970 and 1992.

Psychosis cases

Data on psychiatric admissions were obtained from the Stichting Informatie Gezondheidszorg in the Netherlands (SIG; Foundation for Information on Health Care). The SIG keeps records (with patient anonymity) of admissions to institutions for in-patient psychiatric care in the Netherlands, which are cumulatively updated. Admissions to psychiatric wards of general hospitals are not included. Few cases, however, are likely to have been missed by the SIG register because: (i) these wards represent 9% of psychiatric beds; and (ii) the great majority of psychosis cases are admitted to general psychiatric hospitals (Selten & Sijben, 1994; Neijmeijer & Hutschemaekers, 1995). Information is collected on a standard form that

is completed by the physician in charge of the patient. The SIG returns the form if entries (for example 'ICD-9 diagnosis', 'age', 'sex', 'municipality of birth') have not been completed, thus ensuring that full information will be obtained.

The present analyses refer to the following diagnostic groups: all individuals first discharged from hospital between 1970 and 1992 who had an ICD-9 discharge diagnosis in the categories 295 to 298 (narrow schizophrenia, ICD-9 295.1–295.3, 295.6; broad schizophrenia, ICD-9 295.x and 297.x; affective psychoses, ICD-9 296.x; other psychoses ICD-9 298.x). As there were no qualitative differences in effects between the different diagnostic categories, the category 'all psychosis' (ICD-9 295.x, 296.x, 297.x and 298.x) was also used.

In order to examine the possible effect of misclassification due to diagnostic change after first discharge, the following groups were also examined: (i) all individuals discharged at least twice from hospital between 1970 and 1992 with a discharge diagnosis of schizophrenia (ICD-9 295.x); (ii) all individuals discharged at least twice from hospital between 1970 and 1992 with a discharge diagnosis of affective psychosis (ICD-9 296.x) and never with schizophrenia; (iii) all individuals ever discharged from hospital between 1970 and 1992 and diagnosed with schizophrenia (ICD-9 295.x).

Exposure definition

Two definitions of urbanicity were employed. The first definition involved the CBS 1993 five-level population density rating of Dutch municipalities (Central Bureau of Statistics, 1993a). To ensure sufficiently large groups in each exposure stratum, this rating was collapsed to encompass three levels of urbanicity, the lowest level representing a population density of fewer than 500 addresses per km² and the highest level more than 1500 addresses per km². This three-level definition on the basis of address density will be referred to as the urban exposure.

The second definition of urbanicity comprised birth in the area of the Netherlands with the highest population density. This area includes the three regions of Utrecht, Noord-Holland and Zuid-Holland (the Randstad), where approximately 45% of the Dutch population live on 20% of the country's surface (see also

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above). The mean population density in the Randstad is around 1000 persons per square kilometre, but only 300 persons per square kilometre in the rest of the country (Central Bureau of Statistics, 1993b). This second exposure was included because: (i) the 1993 population density rating may not necessarily be accurate for the environments where individuals were born up to 50 years earlier, whereas the status of the Randstad has been stable over the period under investigation; (ii) place of birth in the Dutch National Case Register was recoded in the 1992 municipality index (because Dutch cities have undergone boundary changes over the period under investigation, so that many of the smaller municipalities have merged with larger cities, the recoding in the 1992 index could potentially lead to an underestimation of any effect of urban birth); (iii) monthly births (necessary to investigate interaction of place and season of birth) were available for the Randstad exposure, but not for the urban exposure.

Time period

For the purpose of the analyses, cohorts were divided into three time periods: 1942/54, 1955/66, 1967/78. Because the maximum follow-up in the oldest cohort was 50 years (born 1942, admitted 1992) but only 25 years in the youngest cohort (born 1967, admitted 1992), which could potentially bias the results (especially if the effect of urban birth was greater in the younger age-at-onset groups), we corrected for differential length of follow-up by also conducting separate analyses where the maximum follow-up in each of the three cohorts was restricted to 20 years (meaning that only cases with onset before the age of 21 years could be included).

Winter birth definition

Guided by previous literature, we defined winter births as births in the months January to March (O'Callaghan *et al.* 1995) and from December to February (Takei *et al.* 1995a). Separate analyses were conducted for these two definitions.

Analyses

Associations between the urban and Randstad exposures on the one hand, and administrative cumulative incidence (hereafter: cumulative in-

cidence – the number of psychosis cases divided by the number of births) of psychotic disorders on the other were expressed as incidence rate ratios (IRR), calculated using the Poisson regression procedure in the STATA statistical program (STATA Corporation, 1995). Interactions between urbanicity on the one hand, and time period and gender on the other were assessed using the likelihood ratio test (LRS). In Poisson regression, the logarithm of the rate is the 'response' variable, and modelled as a linear function of a range of 'explanatory' variables. The exponentiated regression coefficient for a given explanatory variable equals the rate ratio, i.e. the rate in the 'exposed' group divided by the baseline rate.

We also wished to examine the influence of the variable age of onset (defined as age at first admission) on the effect of urbanicity. To estimate the proportion of the Randstad and non-Randstad birth cohorts that would fall into the different age categories, we obtained the population figures for the different age groups in the years 1972, 1982 and 1992 for the Randstad and non-Randstad areas, and applied the proportions of the population falling into these categories to the birth cohorts of the periods 1942–1954, 1955–1966 and 1967–1978 respectively. Cumulative incidences of psychotic disorders were calculated separately for the different age groups in Randstad and non-Randstad areas.

The sum of the Population Attributable Fractions (PAFs) of the second and third stratum of the 1993 urbanicity rating represented the proportion of cases that would have been averted if the risk in all individuals were as low as in those born in the lowest level. Stratum-specific PAFs were calculated as $p'(RR'-1)/RR'$, where p' is the proportion of cases in the stratum, and RR' the stratum-specific IRR.

RESULTS**Urban birth and the risk of later psychosis**

Follow-up of the 1942–78 birth cohorts through the psychiatric case register yielded 42 115 cases of psychosis (47 721 if narrow schizophrenia is considered separate from broad schizophrenia). Both the urban and the Randstad exposures were associated with cumulative incidence of psychosis, regardless of diagnostic category

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Table 1. Association between urban birth and cumulative incidence of psychosis

Diagnosis	Level of urban exposure*			Urban exposure (Linear trend)†	Randstad exposure‡
	1 (Baseline)	2	3		
Schiz. broad					
Cases	2244	5419	9053	16716	
Cum. inc‡	1.3	1.5	2.3		
IRR (95% CI)¶	1.0	1.22 (1.16-1.29)	1.84 (1.75-1.92)	1.39 (1.36-1.42)	1.40 (1.36-1.44)
Schiz. narrow					
Cases	717	1793	3096	5606	
Cum. inc‡	0.4	0.5	0.8		
IRR (95% CI)¶	1.0	1.27 (1.16-1.38)	1.97 (1.81-2.13)	1.44 (1.39-1.50)	1.60 (1.52-1.69)
Aff.					
Cases	1832	3998	5440	11270	
Cum. inc‡	1.0	1.1	1.4		
IRR (95% CI)¶	1.0	1.10 (1.04-1.16)	1.35 (1.28-1.43)	1.18 (1.15-1.21)	1.14 (1.09-1.18)
Other					
Cases	2159	4758	7212	14129	
Cum. inc‡	1.2	1.3	1.8		
IRR (95% CI)¶	1.0	1.12 (1.07-1.18)	1.53 (1.46-1.61)	1.27 (1.24-1.30)	1.08 (1.05-1.12)

* Urban exposure, defined by 1993 municipal population density in three levels.

† The increase in risk per unit increase of the urban exposure variable.

‡ Randstad exposure, defined by birth in densely populated 'Randstad' area v. all other areas.

§ Cumulative incidence per 1000 population.

¶ 95% Confidence interval: interval not including unity denotes statistical significance.

Table 2. Effect of urbanicity* on risk of psychosis (ICD-9 295-298) as a function of age group and birth cohort

Variable	Incidence rate ratio (95% CI)†
Age (years)	
< 21	1.40 (1.33-1.48)
21-35	1.19 (1.16-1.22)
36-50	1.09 (1.05-1.14)
Birth cohort‡	
1942-1954	1.46 (1.29-1.66)
1955-1966	1.54 (1.42-1.66)
1967-1978	1.67 (1.51-1.84)

* Randstad exposure, defined by birth in densely populated 'Randstad' area v. all other areas.

† 95% Confidence interval: interval not including unity denotes statistical significance; two non-overlapping confidence intervals denote statistically significant difference.

‡ Only cases with onset before 21 years to adjust for differential length of follow-up in the different birth cohorts.

(Table 1). The strongest associations existed with narrowly defined schizophrenia (urban exposure IRR linear trend, 1.44, 95% CI 1.39-1.50; Randstad exposure IRR, 1.60, 95% CI 1.52-1.69), followed by broadly defined schizophrenia (urban exposure IRR linear trend, 1.39, 95% CI 1.36-1.42; Randstad exposure IRR, 1.40, 95% CI 1.36-1.44), other psychosis

(urban exposure IRR linear trend, 1.27, 95% CI 1.24-1.30; Randstad exposure IRR, 1.08, 95% CI 1.05-1.12) and affective psychosis (urban exposure IRR linear trend 1.18, 95% CI 1.15-1.21; Randstad exposure IRR, 1.14, 95% CI 1.09-1.18). For (broadly and narrowly defined) schizophrenia, birth in the highest category of the three-level urban exposure increased the risk approximately with a factor two.

The population attributable fractions associated with the urban exposure were: broad schizophrenia, 31%; narrow schizophrenia, 34%; affective psychosis, 16%; other psychosis 21%.

There was an interaction with gender, regardless of diagnostic category (gender interaction term all psychosis, LRS = 10.6 $P < 0.005$), indicating that the effect was around 5% larger for men (IRR urban exposure all psychosis, 1.32, 95% CI 1.29-1.34) than for women (IRR 1.26, 95% CI 1.24-1.28).

Effect of urbanicity over time and in different age of onset groups

The effect of both the urban and the Randstad exposures was progressively greater in younger age groups (Table 2), also if analyses were

*Urbanization and psychosis in The Netherlands*Table 3. *Seasonality, place of birth and risk for schizophrenia (ICD-9 295.x, 297.x)*

Place and season of birth	IRR (95% CI)*		
	Schiz (tot. population)	Schiz (men)	Schiz (women)
Non-Randstad			
Summer	1.0†	1.0†	1.0†
Spring	1.09 (1.0-1.18)	1.10 (0.99-1.22)	1.05 (0.92-1.21)
Winter‡	1.16 (1.06-1.26)	1.11 (1.0-1.23)	1.23 (1.08-1.41)
Autumn	1.04 (0.95-1.13)	1.07 (0.96-1.19)	0.98 (0.84-1.13)
Randstad			
Summer	1.55 (1.43-1.69)	1.60 (1.45-1.78)	1.47 (1.27-1.69)
Spring	1.61 (1.48-1.75)	1.65 (1.49-1.83)	1.53 (1.33-1.76)
Winter‡	1.61 (1.48-1.75)	1.57 (1.41-1.74)	1.69 (1.47-1.93)
Autumn	1.50 (1.38-1.64)	1.60 (1.44-1.77)	1.34 (1.16-1.55)

* 95% Confidence interval: interval not including unity denotes statistical significance.

† Baseline; each of the seven other possible combinations between season and place of birth was compared with the baseline.

‡ Winter defined by period January-March.

conducted in each of the three birth cohorts separately, indicating they were not confounded by the effect of birth cohort described below (Table 2).

The effects of both the urban exposure and the Randstad exposure in the 1942-1978 birth cohorts were significantly modified by time period, regardless of diagnostic category (interaction term broadly defined schizophrenia, LRS = 1.23, $P < 0.001$; other diagnostic categories, all P values < 0.001). For example, IRRs for broadly defined schizophrenia associated with the Randstad exposure (the one that remained stable over the period under investigation) in the earliest, middle and latest cohorts were 1.28 (95% CI 1.23-1.34), 1.42 (95% CI 1.36-1.49) and 1.70 (95% CI 1.52-1.89), respectively. After adjustment for the shorter follow-up period of the latest cohorts (including only cases with onset before age 21 years), similar results were obtained (results for all psychosis adjusted for differential length of follow-up in Table 2).

Alternative definitions of diagnosis

In general, results were similar using groups of cases with discharge diagnoses of schizophrenia and affective psychosis that were made at least twice, and cases with a discharge diagnosis of schizophrenia, ever made. For example, the IRRs linear trend for disorders associated with urban exposure were 1.68 (1.54-1.84; schizophrenia twice discharged), 1.60 (1.51-1.70; schizophrenia ever discharged) and 1.18 (1.01-1.39; affective psychosis twice discharged).

As in the analyses with first discharge diagnoses, effect sizes increased with earlier age at onset and later time period.

Interaction between seasonality and place of birth

There was no significant interaction between the Randstad exposure and winter birth (however defined). Inspection of the results in the total sample revealed that the risk for broadly defined schizophrenia for both urban- and rural-born individuals was highest for those born in winter, although this trend appeared to be very weak (Table 3). A stronger trend could be observed in women, but there was no evidence for a synergistic effect of urban birth.

DISCUSSION

Risk for psychotic disorder was positively and linearly associated with the degree of urbanicity of place of birth. Associations were most prominent for, but not specific to, schizophrenia. Individuals born in the highest category of a three level urban exposure were around twice as likely to develop schizophrenia. The effect of urban birth was greatest for illness in: (i) individuals from the most recent birth cohorts; (ii) men; and (iii) early onset cases. Results concerning possible interaction between season and place of birth were inconclusive.

Limitations

The results of the study are based on patients with psychiatric disorders admitted to hospital. Although not all cases of schizophrenia are

admitted to hospital during the first episode, many of these will eventually become in-patients and thus subsequently become registered. The findings regarding affective psychosis, however, cannot be generalized to all affective disorders, especially the milder forms, as not all individuals with such illnesses will receive psychiatric treatment, let alone be admitted to hospital.

In theory, large cities and densely populated areas could have more psychiatric beds, lowering the admission threshold for individuals with psychosis and thus leading to a spurious increase in administrative incidence relative to rural areas. However, 1992 CBS figures (Central Bureau of Statistics, 1993b) show that the number of psychiatric beds in the Randstad (166 per 100 000 population) was similar to that of the rest of the Netherlands (158 per 100 000 population). Figures from 1971 show a similar picture (Randstad, 199 beds per 100 000; Non-Randstad, 203 per 100 000). CBS figures also show that bed occupancy rates were similar for both areas (around 95%). It is therefore unlikely that our results were biased by differential bed availability or changes therein over time.

Admissions to psychiatric wards in the general hospital (PWG) are not included in the Case Register. PWG beds, although few, are somewhat less numerous in the Randstad area (Randstad 7.1 per 100 000 population; outside Randstad, 13.3 per 100 000 population), which could constitute a bias. However, PWG capacity represents only 9% of all general psychiatric beds, and around two-thirds of the patients in PWG settings are women admitted for short periods with non-psychotic disorders (Neijmeijer & Hutschmaekers, 1995). Only 5% of patients are diagnosed with schizophrenia (psychiatric hospitals, 16%) many, if not most, of whom will eventually become registered in the National Case Register through subsequent admissions in the psychiatric hospitals (Neijmeijer & Hutschmaekers, 1995). Thus, even if we assume that we missed as much as 5% of all schizophrenia cases, and taking into account the somewhat uneven spread of PWG beds, the rate ratio for the Randstad exposure in schizophrenia would only be slightly reduced (RR, 1.33; 95% CI, 1.29–1.37).

The distance to psychiatric facilities has been reported to be inversely related to admission rates (Jarvis, 1851). The effect of distance,

however, is small for severe psychiatric disorders (Davey & Giles, 1979), and in a small country like the Netherlands, distance to psychiatric facilities is unlikely to be an issue for any psychiatric disorder. A previous study in the Netherlands found no effect of distance to psychiatric facility on admission rates (Sytema, 1991).

Social networks may be better developed in rural areas, or attitudes towards psychiatric institutions more negative, resulting in a larger proportion of individuals with psychosis being treated on an out-patient basis. It is unlikely, however, that admission of a patient with severe psychiatric disorder such as schizophrenia would be more than delayed by differential attitudes. Furthermore, even if different attitudes did contribute to the reported effect, it: (i) is unlikely to explain the 97% excess risk for narrowly defined schizophrenia associated with birth in the most urbanized environments (Table 1); (ii) cannot explain why the urban birth effect increased over time (if anything, one would expect differences between 'traditional' rural and urban attitudes to decrease with time), and (iii) cannot explain the variation of the urban birth effect with age of onset and gender.

Although the urban birth effect precludes a mechanism of pre-morbid drift to the cities of genetically predisposed individuals, parental choice to reside in urban areas may still be influenced by the same genes that predispose to psychiatric illness in the offspring. Rutter and colleagues (1975), however, noted that psychiatric disorders were much more frequent in urban areas, even when using geographically stable samples of children born and bred in the city, of parents born and bred in the city. Their investigation focused on non-psychotic disorders in children, and this type of correlation between genetic predisposition and urban environment may play a more important role in adult-onset psychotic illness. It is unlikely, however, that differential parental geographical mobility could have explained the full excess risk associated with urban birth, demonstrated in this study. Furthermore, even in the case of differential urban in-migration of parents carrying psychosis genes, the city environment could still be of aetiological importance in the subsequent expression of psychotic symptoms as discussed below.

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There is evidence of an increased incidence of schizophrenia in Surinamese and Dutch Antillean immigrants to the Netherlands (Selten *et al.* 1997). However, large-scale migration from these countries started in 1973 and most children born to immigrants were too young to develop psychosis in the period 1970–1992. Therefore, our results were not confounded by ethnic group.

In the analyses on modification of the urbanicity effect by age, the proportion of individuals in the birth cohorts who would fall later into the various age categories had to be estimated using population figures. We are confident that this estimation procedure was adequate because the age categories constituted large sections of the population with only minimal differences between urban and rural areas. Consequently, such differences (which would affect the denominator), even if they were much larger, are unlikely to have substantially affected the cumulative incidences in Randstad and non-Randstad areas and could not explain the decrease in the urbanicity effect in the older age groups.

Interpretation*Urban birth versus urban residence*

As urban birth and urban residence are likely to be highly correlated, neither our own study nor any of the earlier cited studies (Torrey & Bowler, 1990; Lewis *et al.* 1992; Takei *et al.* 1992; Freeman, 1994) can exclude the possibility that the associations that were found in fact reflect associations between urban life and risk for mental illness. For example, in our sample of patients around three-quarters of individuals born in municipalities with the highest level of urban exposure, were also living in such municipalities at the time they became ill. To distinguish between an urban birth effect and an urban residence effect one would need the corresponding population data of individuals born in urban areas who subsequently moved to rural regions and vice versa. No such data are available in the Netherlands.

Although we have no formal way of testing whether an urban risk factor affected vulnerable individuals around the time of birth or around the time of illness onset, the fact that effect sizes were smaller for affective psychosis may point to the former rather than the latter. For example,

there is evidence that the onset of affective disorder is more strongly related to environmental adversity such as life events than is schizophrenia (Paykel, 1978; Bebbington *et al.* 1993). Therefore, if the high rates of disorder in urban environments were to be explained on the basis of high-risk groups experiencing more psychosocial stress around the time of illness onset, one would have expected a stronger effect of urban exposure on the rate of affective psychosis than on the rate of schizophrenia. On the other hand, our results were inconclusive with regard to the possible interaction between season and place of birth, the demonstration of which would have supported an urban birth rather than an urban residence effect. Our failure to demonstrate such an interaction may be related to the fact that the Randstad exposure did not provide enough contrast between exposed and non-exposed individuals. A previous analysis within the patient group did suggest such an interaction in women (Van Os *et al.* 1996).

Interaction with demographic variables

Because it is unlikely that the genetic make-up of individuals living in urban and rural environments in the same small country differ from each other, the urban risk factor is likely to be environmental in nature. As it is implausible that environmental factors are sufficient to cause schizophrenia and related psychoses in the absence of genetic predisposition (Sham, 1996), a model of interaction can be envisaged where genetically predisposed individuals run a higher risk of developing illness in adverse urban environments operating either around the time of birth or at the time of illness onset. For example, an excess of stressful social precipitants associated with residence in urban environments (Brown & Prudo, 1981) may interact with genetic predisposition to 'bring forward' the age at onset in vulnerable individuals (Brown *et al.* 1973). Population surveys in the Netherlands have shown a higher prevalence of stress, divorce, noise pollution, crime and poor perceived mental and physical health among Dutch people living in urban areas (CBS, 1993a; Bijl *et al.* 1997; Rijksinstituut voor Volksgezondheid en Milieu, 1997), supporting the notion that an excess of social precipitants contributes to the higher incidence of psychosis

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in urban areas. Other factors, such as high levels of cannabis use by Dutch urban adolescents (Kuipers *et al.* 1993; Korf *et al.* 1995) may also play a role. Alternatively, early factors associated with urban birth that affect brain development such as prenatal exposure to viral infections (Takei *et al.* 1995b), nutritional deficiency (Brown *et al.* 1995; Susser *et al.* 1995) or exposure to pollutants such as lead may also give rise to an increase in risk for adult mental illness.

Male sex and early onset both predict poorer prognosis in schizophrenia (Navarro *et al.* 1996), possibly because of greater associated developmental impairment (Castle & Murray, 1991; Murray *et al.* 1992). Thus, the fact that the effect of urbanicity was greatest in men and in the younger age groups suggests that the risk factors of which urban birth (or urban residence) is a proxy indicator may interact with, or cause increased expression of, developmental vulnerability for adult mental illness.

Our finding that the relative risk of urban birth increased with time could be due to a true effect size increase in those exposed to urban birth, associated with rising levels of, and rising contrasts in urbanization in the Netherlands. An alternative explanation, however, is that of a time-related decrease in the cumulative incidence in the groups not exposed to urban birth. Because Case Register data based on hospital admissions are suitable to examine relative risks, but less suitable to examine absolute risks (incidence) and changes therein (Van Os *et al.* 1993), our data cannot shed more light on this issue. Thus, whether the increase in cumulative incidence associated with urban birth is relative, absolute or both must be the subject of further investigations. If, however, there has been a true decline in the incidence of schizophrenia as suggested (Takei *et al.* 1996), it may be concluded that the decline was not due to a fall in the prevalence of the environmental risk factor associated with urban environments.

The fact that no qualitative, but only quantitative differences between diagnostic categories were found is in line with studies on other risk factors for schizophrenia that are present before disease onset such as ventricular enlargement (Elkis *et al.* 1995) and developmental deviance (Van Os *et al.* 1997a), and suggest that these different disorders may lie on a severity con-

tinuum associated with differential degree of exposure to genetic and other risk factors (Van Os *et al.* 1997b).

The presence of dose-response relationships between level of urbanicity and cumulative incidence of schizophrenia is compatible with a causal (Hill, 1965) relationship and, therefore, possibilities for prevention. Whatever the window of exposure and the exact mechanism involved, the public health importance of the urban risk factor may be considerable, as indicated by a population attributable fraction of around 30% for (broadly and narrowly defined) schizophrenia. Therefore, the possible aetiological mechanism associated with urban environments, as well as the exact window of exposure, urgently warrant further investigation.

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CHAPTER 4

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Urbanization and risk for schizophrenia: does the effect operate before or around the time of illness onset?

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ABSTRACT

Background. Higher level of urbanicity of place of birth and of place of residence at the time of illness onset has been shown to increase the risk for adult schizophrenia. However, because urban birth and urban residence are strongly correlated, no conclusions can be drawn about the timing of the risk-increasing effect. The current study discriminated between any effect of urbanization before and around the time of illness onset.

Methods. All individuals born between 1972 and 1978 were followed up through the Dutch National Psychiatric Case Register for first admission for schizophrenia until 1995 (maximum age 23 years). Exposure status was defined by a combination of place of birth and place of residence at the time of illness onset in the three most densely populated provinces of the Netherlands (the 'Randstad', exposed) or in all other areas (the 'non-Randstad', non-exposed). The risk for schizophrenia was examined in four different exposure groups: non-exposed born and non-exposed resident (NbNr, reference category), non-exposed born and exposed resident (NbEr), exposed born and non-exposed resident (EbNr) and exposed born and exposed resident (EbEr).

Results. The greatest risk for schizophrenia was found in the EbNr group, without evidence for any additive effect of urban residence (rate ratio (RR) for narrow schizophrenia in EbNr group, 2.05 (95% CI 1.18–3.57); in EbEr group, 1.96 (95% CI, 1.55–2.46)). Individuals who were not exposed at birth, but became so later in life, were not at increased risk of developing schizophrenia (RR for narrow schizophrenia in NbEr group, 0.79 (0.46–1.36)).

Conclusion. The results suggest that environmental factors associated with urbanization increase the risk for schizophrenia before rather than around the time of illness onset.

INTRODUCTION

The association between urban residence, urban upbringing and urban birth on the one hand, and later schizophrenia on the other has been well established (Eaton, 1974; Lewis *et al.* 1992; Takei *et al.* 1992; Freeman, 1994; Kendler *et al.* 1996; Torrey *et al.* 1997; Verdoux & Takei,

1997; Marcelis *et al.* 1998). Because urban birth/upbringing is strongly associated with later urban residence, it is not known whether the risk-increasing mechanism involves an early effect (urban birth/upbringing), a late effect (urban residence around the time of onset) or both. Early effects may include environmental factors that interfere with brain development and enhance the susceptibility for psychosis in adult life (Torrey & Bowler, 1990; Lewis *et al.* 1992; Takei *et al.* 1995). Later effects may include, for example, a higher prevalence of stressful life events and chronic difficulties in

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urban areas (Brown & Prudo, 1981). In order to determine the timing of any risk-increasing mechanism, we examined ways in which to discriminate between the effects of urban birth and urban residence at the time of first admission. Because schizophrenia is associated with risk factors that operate early in life (Jones *et al.* 1994), as well as with risk factors that have an impact around the time of onset (Brown & Birley, 1968), we hypothesized the existence of both an early and a late effect.

METHOD

Exposure definition

The definition of urbanicity consisted of birth and/or 'adult' residence in the area of the Netherlands with the highest population density and has been described in detail in a previous report (Marcelis *et al.* 1998). The urban exposure comprises an area including the three provinces of Utrecht, Noord-Holland and Zuid-Holland (the 'Randstad'). The Randstad constitutes a 'megapolis' of 6.7 million people (45% of the Dutch population) who live on 20% of the country's surface. All areas in the Netherlands outside the Randstad (the nine other provinces) are hereafter referred to as the 'non-Randstad'. The stark contrast in population density between Randstad and non-Randstad areas has always existed, making the exposure time-invariant (Marcelis *et al.* 1998).

Schizophrenia cases

Data on psychiatric admissions were obtained from the Stichting Informatie Gezondheidszorg in the Netherlands (SIG; Foundation for Information on Health Care). The SIG keeps anonymized records of all admissions to institutions or in-patient psychiatric care in the Netherlands, cumulatively updated through a linkage procedure. Admissions to psychiatric wards of general hospitals are not included. A small proportion of cases is therefore likely to have been missed by the SIG register as these wards represent 9% of psychiatric beds. However, the great majority of psychosis cases are admitted to general psychiatric hospitals (Selden & Sijben, 1994; Neijmeijer & Hutschemaekers, 1995). Information is collected on a standard form that is completed by the physician in

charge of the patient. The information obtained includes age, sex, ICD-9 (World Health Organization, 1978) diagnosis, place of birth and place of residence. The SIG returns the form if entries have not been completed, thus ensuring that full information is obtained. All individuals aged 16 years or older at first discharge from hospital between 1972 and 1995 who had an ICD-9 discharge diagnosis 295.x and 297.x (broad schizophrenia) were included in the analyses. In addition, narrow schizophrenia (ICD-9 discharge diagnosis 295.1-295.3, 295.6) was considered separately from broad schizophrenia.

For the purpose of the analyses, four exposure groups were defined, based on whether individuals had been exposed (E) or not (N) to the urban environment either at the time of birth or at the time of first admission. These four groups were: (i) neither exposed at birth, nor exposed at admission (NbNr, reference category); (ii) not exposed at birth, exposed at admission (NbEr); (iii) exposed at birth, not exposed at admission (EbNr); (iv) exposed at birth, exposed at admission (EbEr).

Birth cohorts

While the cases could be readily classified into the four exposure groups, classification of the population (birth cohorts) required a different procedure. We calculated, for all births in Randstad and non-Randstad areas between 1972 and 1978, the rate of change in exposure status by age 23 years (the maximum age of the cases: born 1972, admitted 1995). This was done with the help of internal migration data in the Netherlands obtained from the Dutch governmental Central Bureau of Statistics (CBS). Data on internal migration per province and age group were available from 1972 onwards, constituting the starting point of the follow-up.

First, the CBS provided figures on all yearly live births recorded between 1972 and 1978 in any of the 12 Dutch provinces. Secondly, we obtained information on internal migration (i.e. the change in exposure status) by age (5-year age groups) in the general Dutch population for the years 1972, 1977, 1982, 1987, 1992 and 1996. The figures for the intervening years were interpolated assuming a linear change over the 5-year periods. For example, the rate of change in exposure status for individuals aged 0-4 years

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born in the Randstad area was 0.164% in 1972. In 1977, when these individuals were aged 5-9 years, this was 0.139%. The rate over the intervening years was computed: for 1973, $0.164 + 0.2 \cdot (0.139 - 0.164) = 0.159$; for 1974, $0.164 + 0.4 \cdot (0.139 - 0.164) = 0.154$; for 1975, $0.164 + 0.6 \cdot (0.139 - 0.164) = 0.149$; and for 1976, $0.164 + 0.8 \cdot (0.139 - 0.164) = 0.144$. The cumulative rate for change in exposure status by age 23 years was obtained by summing up the rates of all the individual years.

Because the data on internal migration were available by 5-year age-groups, individuals who did not reach the minimum age of 16 and were unlikely to be 'at risk' for schizophrenia before 1996, could not be excluded from the denominator with exactitude for all the years. For example, of the individuals aged 0-4 years in 1982, only those aged 3 or 4 years in 1982 would have reached the minimum age at risk by 1995. To examine possible bias due to this lack of precision we constructed denominator data that either included all individuals of this age group (i.e. including the 0, 1 and 2 years-old in 1982 - the 'inclusive' approach) or excluded them all (the 'exclusive' approach). Thus, we used both 'extreme' denominators in separate analyses, the former leading to relatively underestimated and the latter to relatively overestimated rates of schizophrenia.

The calculation of cumulative rate of change in exposure status of birth cohorts was based on two assumptions. The first assumption was that the rate of population mutation due to death or external migration before the age of 24 years would be independent of the exposure. In order to check this assumption, we obtained mortality and emigration rates by province and age group for the years 1986, 1991 and 1996 to determine whether these rates varied with the level of exposure.

The second assumption was that the rate of moving back after change of exposure status would be small and similarly independent of the exposure. Any misclassification occurring in the EbNr and NbEr groups would affect the number of individuals in all four exposure categories. For example, some individuals classified as EbNr might have moved back so that their exposure status in reality was EbEr. Such a misclassification in the birth cohorts could lead to a spurious reduction of any urban birth effect,

because in the calculation of the rate ratio with the reference category (EbNr rate divided by NbNr rate), the EbNr rate would be spuriously low because of the inflated denominator. Similarly, a proportion of NbEr individuals could consist of misclassified NbNr individuals, leading to a spurious reduction of the urban residence effect. Thus, in order to check how violations of the second assumption might have influenced the results we recalculated risks for schizophrenia assuming varying rates of misclassification of individuals in the NbEr- and the EbNr-group.

Analyses

Associations between the four categories of exposure and cumulative incidence of schizophrenia, were expressed as rate ratios (RR), calculated using the Poisson regression procedure in the STATA statistical programme (STATA Corporation, 1995). In Poisson regression, the logarithm of the rate is the 'response' variable, and modelled as a linear function of 'explanatory' variables. The exponentiated regression coefficient for a given explanatory variable equals the rate ratio, i.e. the rate in the 'exposed' group divided by the baseline rate.

RESULTS

The risk for schizophrenia was increased in individuals who were exposed to an urban environment (Randstad) at birth, regardless of exposure status at the time of illness onset: broad schizophrenia - RR EbNr-group, 2.29 (95% Confidence Interval (CI), 1.64-3.22), RR EbEr-group, 1.67 (95% CI, 1.44-1.95); and narrow schizophrenia - RR EbNr-group, 2.05 (95% CI, 1.18-3.57), RR EbEr-group, 1.96 (95% CI, 1.55-2.46). Thus, there was no additive effect of urban residence in those exposed at birth. No statistically significant association was found between a non-Randstad place of birth combined with a later Randstad residence on the one hand, and risk for schizophrenia on the other: broad schizophrenia - RR NbEr-group, 0.98 (95% CI, 0.72-1.34); narrow schizophrenia - RR NbEr-group, 0.79 (95% CI, 0.46-1.36) (Table 1).

The above results apply to the 'inclusive' approach in calculating the denominator (in-

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Table 1. *Effects of level of urbanicity at the time of birth and at the time of first admission on the cumulative incidence of early onset* schizophrenia per 1000 live births*

	Exposure-status†			
	NbNr	NbEr	EbNr	EbEr
Cases of schizophrenia (broad definition)‡, N	284	45	38	399
Births	656446	105978	38299	550914
RR (95% CI)	1	0.98 (0.72-1.34)	2.29 (1.64-3.22)	1.67 (1.44-1.95)
P	—	0.91	< 0.001	< 0.001
Cumulative incidence	0.4	0.4	1.0	0.7
Cases of schizophrenia (narrow definition)‡, N	117	15	14	192
Births	656446	105978	38299	550914
RR (95% CI)	1	0.79 (0.46-1.36)	2.05 (1.18-3.57)	1.96 (1.55-2.46)
P	—	0.40	< 0.02	< 0.001
Cumulative incidence	0.2	0.1	0.4	0.4

* Age < 24 years.

† Randstad exposure, defined by birth or residence in densely populated 'Randstad' (exposed) v. birth or residence in all other areas (non-exposed); NbNr group = non-exposed birth and non-exposed residence (reference category); NbEr group = non-exposed birth and exposed residence; EbNr group = exposed birth and non-exposed residence; EbEr group = exposed birth and exposed residence.

‡ Broad schizophrenia, ICD 295.x, 297.x; narrow schizophrenia, ICD 295.1, 295.3, 295.6.

Table 2. *Assumption testing: effects of possible misclassification of birth cohort exposure groups*

Proportion of misclassified* individuals			Exposure status†			
NbEr (%)	EbNr (%)		NbNr (reference)	NbEr	EbNr	EbEr
20	20	RR (95% CI)‡	1.0	1.03 (0.60-1.75)	2.65 (1.52-4.61)	1.99 (1.58-2.51)
		P	—	0.93	< 0.001	< 0.001
20	10	RR (95% CI)	1.0	1.03 (0.60-1.75)	2.35 (1.35-4.10)	2.01 (1.59-2.52)
		P	—	0.93	< 0.002	< 0.001
20	5	RR (95% CI)	1.0	1.03 (0.60-1.75)	2.23 (1.28-3.88)	2.01 (1.60-2.53)
		P	—	0.93	< 0.005	< 0.001
30	5	RR (95% CI)	1.0	1.19 (0.70-2.04)	2.26 (1.30-3.94)	2.04 (1.62-2.57)
		P	—	0.53	< 0.004	< 0.001
40	5	RR (95% CI)	1.0	1.41 (0.82-2.41)	2.30 (1.32-4.00)	2.07 (1.65-2.61)
		P	—	0.21	< 0.003	< 0.001
50	5	RR (95% CI)	1.0	1.72 (1.00-2.94)	2.33 (1.34-4.06)	2.11 (1.67-2.65)
		P	—	0.05	< 0.003	< 0.001

* Misclassification = the proportion of EbNr individuals that should have been classified EbEr (EbNr group) and the proportion of NbEr individuals that should have been classified NbNr (NbEr group).

† Randstad exposure, defined by birth or residence in densely populated 'Randstad' (exposed) v. birth or residence in all other areas (non-exposed); NbNr group = non-exposed birth and non-exposed residence (reference category); NbEr group = non-exposed birth and exposed residence; EbNr group = exposed birth and non-exposed residence; EbEr group = exposed birth and exposed residence.

‡ RRs apply to narrow schizophrenia: ICD 295.1-295.3, 295.6.

clusion of some individuals that were not yet 'at risk' for schizophrenia by 1995 as explained in the Method section). The 'exclusive' approach gave similar results: broad schizophrenia - RR EbNr group, 2.95 (95% CI, 2.10-4.13) and RR EbEr group, 1.70 (95% CI, 1.46-1.98) and RR NbEr group, 1.22 (95% CI, 0.89-1.67); narrow schizophrenia - RR EbNr group, 2.64 (95% CI, 1.51-4.59), RR EbEr group, 1.98 (95% CI, 1.58-2.50) and RR NbEr group, 0.99 (95% CI, 0.58-1.69).

Assumption testing

The mortality rates (total deaths per 1000 population) up to age 24 years did not vary with the level of exposure (in 1986, the mortality rate per 1000 in the Randstad was, 0.65 and in the non-Randstad, 0.64; in 1991 these rates were 0.61 and 0.58; and in 1996 0.50 and 0.54, respectively). The emigration rates up to age 24 were slightly higher in Randstad than in non-Randstad areas (rates per 1000 for the Randstad

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and non-Randstad respectively were: 4.9 and 3.3 in 1986; 4.8 and 3.6 in 1991; 4.9 and 3.9 in 1996. These differences were, nevertheless, too small to produce any substantial alterations in the relevant effects (results after adjustment for external migration based on rates of 1986: narrow schizophrenia, RR EbNr group, 2.05; RR EbEr group, 1.96; and RR NbEr group, 0.80).

We tested how the results would change under the assumption of varying rates of misclassification in the EbNr and NbEr birth cohorts. In general, results were only slightly affected (Table 2). Only if the misclassification rate was assumed to be (i) extremely high, and (ii) ten times higher in the NbEr-group than in the EbNr-group (50% v. 5% respectively), then a statistically significant urban residence effect in the NbEr group could be demonstrated. Even then, however, there was no increased risk in the EbEr group compared to the EbNr group, showing no additive urban residence effect (results for narrow schizophrenia: RR EbNr-group, 2.33 (1.34-4.06); RR EbEr-group, 2.11 (1.67-2.65); RR NbEr-group, 1.72 (1.00-2.94)). Higher rates of misclassification in the EbNr-group compared to the misclassification rates in the NbEr-group, led to an even more accentuated urban birth effect, again without showing an (additive) urban residence effect (results not shown).

DISCUSSION

The present study examined the effect of level of urbanicity of place of birth/upbringing and that of later residence on the risk for schizophrenia in individuals up to age 23 years. The results showed that having been born or brought up in densely populated areas strongly increased the risk for subsequent schizophrenia. Our data did not provide evidence for an effect of urban residence at the time of first admission, regardless of whether individuals were born in exposed or in non-exposed areas.

Methodological issues

The results were based on in-patients with a diagnosis of schizophrenia. Although not all cases of schizophrenia are admitted to hospital during the first episode, many of these will eventually become ill enough to be admitted and thus subsequently registered in the Case Register.

As the analyses were restricted to cases with a maximum age of 23 years, the present results may not be generalizable to the total population of schizophrenia patients. However, in our previous study (Marcelis *et al.* 1998) we found that the effect of urban birth was most prominent in early onset cases (age < 21 years), which appears to justify restriction to a group with early age of onset.

Any effect of differential moving back after change of exposure status in the various birth cohorts did not influence the results prominently. Under all circumstances, the greatest risk was found in the EbNr group (exposed birth, non-exposed residence at the time of admission). Only in extreme and highly improbable situations was there evidence for an urban residence effect in non-exposed born individuals (i.e. NbEr group).

Because the rates of schizophrenia have been shown to be increased in Surinamese and Dutch Antillean immigrants to the Netherlands (Selten *et al.* 1997), the present results could, in theory, have been confounded by ethnic group. However, large-scale migration from these countries only took place from 1973 onwards, so that only a small group of the oldest among the second generation of these ethnic groups could have had any influence on the results.

It could be argued that the 'province definition' of urbanicity (Randstad v. non-Randstad areas) provided less contrast between exposed and non-exposed areas compared to definitions of urbanicity at the level of cities. In our previous study, however, results were similar regardless of whether the province definition or a three-level municipality population density rating was used. In addition, the province definition is more time invariant and much less sensitive to bias due to changes in residence in the years preceding admission to hospital.

The present investigation is limited by the fact that the exact point of change in exposure status is unknown; the change in exposure status could have occurred at any time between birth and first hospital admission. Continuous registration of all individual migration patterns in both the cases and the general population would have been needed to assess the exact timing of the exposure. Such complex data, however, are not collected in the Netherlands, and whether the effect of urban 'birth' is confined to the pre- or

peri-natal period, or extends into the rearing period and early adolescence or even later, consequently remains unanswered. The findings, nevertheless, strongly argue against a risk increasing mechanism around the time of illness onset.

Findings

The results agree with the suggestion by Lewis and colleagues (1992) that environmental factors associated with urbanization influence the risk for schizophrenia early during the life-span and before illness onset, rather than at the time of illness onset. Early risk factors associated with urban birth/upbringing may include prenatal exposure to viral infections (Takei *et al.* 1995), nutritional deficiency (Brown *et al.* 1995; Susser *et al.* 1995), stressful life events (Brown & Prudo, 1981), cannabis use (Korf *et al.* 1995), head trauma (Wilcox & Nasrallah, 1987), and exposure to pollutants such as lead.

Dutch population surveys have shown a higher prevalence of stressful factors such as noise pollution, feeling unsafe, divorce, crime and poor perceived mental and physical health in urban areas (Bijl *et al.* 1997; Rijkinstituut voor Volksgezondheid en Milieu, 1997). As it is unlikely that environmental factors are sufficient to cause schizophrenia in the absence of genetic predisposition (Sham, 1996), psychosocial factors may precipitate illness in predisposed individuals or interact with genes in other ways (Van Os & Marcelis, 1998).

Noise-pollution in urban areas may lead to (chronic) stress conditions and interfere with development of normal brain functioning and behaviour. Physical hazards and demands on the job ('noisy working conditions') have been found to be associated with increased risk of psychotic disorders (Link *et al.* 1986; Muntaner *et al.* 1991). Link and colleagues (1986) noted that patients with a diagnosis of schizophrenia significantly more often experienced noise pollution than healthy community controls at the time of their first full-time jobs (prior to illness onset), which could not be explained by a process of social selection. This finding agrees with recent evidence indicating an increased dopamine turnover and impaired prefrontal cortical cognitive function consequently upon exposure to noise stress in monkeys (Arnsten & Goldman-Rakic, 1998). Use of

cannabis in Dutch cities is high and has even further increased during the last decade; among school children as young as 10 years of age, cannabis use is not uncommon (Kuipers *et al.* 1993). This pattern of chronic drug use may also interfere with or contribute to the vulnerability for schizophrenia (Andreasson *et al.* 1987).

In conclusion, several mechanisms could explain the association between schizophrenia and level of urbanicity of place of birth and upbringing we observed. As it has been suggested that the effect of urbanicity is increasing over time (Marcelis *et al.* 1998), more information on the nature of the presumed aetiological mechanisms is needed.

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Obstetric Complications and Familial Morbid Risk of Psychiatric Disorders

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Obstetric complications (OCs) have been found to occur in higher frequency in patients with schizophrenia. One explanation for this finding is that the genes that contribute to the schizophrenia phenotype also influence the likelihood to experience OCs. If this were true, morbid risk of psychiatric illness should be higher in the first-degree relatives of both schizophrenic and control probands exposed to OCs, compared to probands not exposed to OCs. We set out to test this hypothesis.

Information on OCs, blind to family history of psychiatric disorder, was collected retrospectively through maternal interview in 151 psychotic patients and 100 controls. Family history (FH) in relatives of cases ($n = 600$) and controls ($n = 461$) was assessed with the FH-RDC and through personal interviews. Tests for associations between family history and OCs were conducted using Cox proportional hazard regression. In the cases, familial morbid risk of affective disorder was greater in those with a history of OCs (hazard ratio (HR) = 1.9, $P = 0.007$). Analyses examining individual complications revealed associations between FH of affective disorder and pre-eclampsia (HR = 2.9, $P = 0.003$) and FH of affective disorder and breech presentation (HR = 2.8, $P = 0.02$), especially when family history in the relatives was confined to affective illness in the mother (HR pre-eclampsia = 4.4, $P = 0.009$; HR breech-presentation = 4.2, $P = 0.008$). In controls, affective illness in the mother was not only associated with breech presentation (HR = 7.0, $P = 0.01$) and pre-eclampsia (HR = 4.4, $P = 0.03$) but also with other complications. Familial morbid risk of schizo-

phrenia and related psychoses was not associated with OCs.

The positive associations between OCs and familial morbid risk of affective disorder suggest that the factors that contribute to familial aggregation of affective symptoms in psychotic patients also influence the likelihood to experience OCs. Although the proportion of OCs that could be attributed to these factors was very small, part of the relationship between family history of affective disorder and psychosis may be mediated by OCs. *Am. J. Med. Genet. (Neuropsychiatr. Genet.)* 81:29-36, 1998.

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KEY WORDS: psychosis; genetics; genotype-environment correlation; genotype-environment interaction; family history

INTRODUCTION

The importance of genes in the causation of schizophrenia and related disorders has been demonstrated by family, twin, and adoption studies [Gottesman and Shields, 1982; McGuffin et al., 1984, 1994]. On the other hand, discordance rates for psychosis as high as 50% in monozygotic twins suggest that environmental factors are also involved in aetiology.

Possible environmental factors such as recent life stresses and pre- and perinatal complications are usually considered separately from genetic influences in scientific studies. However, environmental factors may be associated with genes in several ways [Plomin, 1994]. For example, there is evidence that genetic and early constitutional factors make some individuals more sensitive to the influence of life events, thereby increasing their risk, for example, of later depressive illness (genotype-environment interaction or stress-vulnerability model [Rogers, 1991; Kendler et al., 1995]). On the other hand, genetic factors may also influence the likelihood of exposure to life events, for

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example, through certain personality traits (genotype-environment correlation model [Kendler, 1995; Champion et al., 1995; Saudino and Plomin, 1996]).

Thus, *interaction* between genes and environment refers to the possibility that genes influence the individual's sensitivity to the environmental risk factor. In the absence of interaction, cases of illness caused by environmental risk factors alone are "phenocopies" of genetic cases. *Correlation* between genotype and environment implies genetic control of exposure to the environment, positive correlation indicating increased risk of exposure, and negative correlation indicating decreased risk of exposure. Interaction and correlation are independent, and both may be operating at the same time.

There has been some work on the possible relationship between genetic factors and obstetric complications (OCs) in the causation of psychosis. Previous studies have concurred as regards the likelihood of genotype-environment interaction [Parnas et al., 1982; Mednick et al., 1987; Cannon et al., 1993]. For example, Cannon and colleagues [1993] defined three categories of genetic exposure on the basis of the presence of schizophrenia and related disorders in neither, one, or two parents. Ventricular enlargement was examined in these three groups in relation to OCs. It was shown that the association between OCs and ventricular enlargement was stronger in individuals at high genetic risk than in individuals at low genetic risk. These findings suggest that the expression of vulnerability to schizophrenia may be at least in part contingent on the additional presence of OCs.

Genotype-environment correlation in relation to OCs, however, has been less researched. One prediction of genotype-environment correlation would be that births to parents with schizophrenia have an increased risk of obstetric complications. The two reviews considering this question, however, reached different conclusions [McNeil, 1991; Sacker et al. 1996]. Furthermore, as there is 1) evidence suggesting genetic overlap between schizophrenia and affective disorder [Taylor et al., 1993; Maier et al., 1993; Farmer et al., 1987] and 2) affective disorder has been shown to adversely affect pregnancy outcomes [Hedegaard et al., 1993; Orr and Arden Miller, 1995], it may be useful to also consider, within the category of psychosis, correlations between OCs and affective diathesis.

In this study, we wished to examine the possibility of genotype-environment correlation; that is, whether genetic factors associated with schizophrenia increase the likelihood of exposure to obstetric complications. Although it is difficult to directly assess correlations between genetic factors and OCs in psychotic illness, it is quite feasible to examine the predictions of the correlation model in relation to familial morbid risk in psychotic probands and control probands. Since the cause of familial aggregation of schizophrenia and affective psychosis is largely genetic [McGuffin et al., 1994], familial morbid risk may serve as an indicator of the degree of genetic liability. It can be shown that if familial morbid risk is positively associated with OCs in both psychotic probands and control probands, genes and OCs must be positively correlated, regardless of

the concurrent pattern of interaction (Appendix). This pattern of expected familial morbid risks is depicted in Figure 1 and will be examined hereafter in the first-degree relatives of samples of 1) psychotic probands, and 2) well controls.

MATERIALS AND METHODS**Index Cases/Control and Baseline Assessment**

Details on the method and sampling of the study have been described previously [Harvey et al., 1990; Jones et al., 1993; Van Os et al., 1996]. In brief, all patients age 16 to 60 years admitted to two South London hospitals with at least one psychotic symptom according to the Research Diagnostic Criteria (RDC) of Spitzer et al. [1978] were identified. Attempts were made to interview patients meeting these criteria within three days of admission. Baseline assessments comprised the Present State Examination [PSE; Wing et al., 1974] and detailed demographic and historical information. Cases were included if, in addition to their psychotic symptoms, they fulfilled the RDC criteria for schizophrenia, schizo-affective disorder, bipolar disorder, manic disorder, major depression, or unspecified functional psychosis ($n = 337$). Additional data on family history and obstetric complications through maternal interview were available for 151 of these subjects. All subjects participating in the study provided written informed consent, conforming to the local ethics' committee guidelines.

Walk-in attenders at the Accident and Emergency Department of King's College Hospital—a large general hospital in the same catchment area—were identified as representative of the general population age 16–60 years who would be admitted to any of the three index psychiatric hospitals if they became psychotic. Of 330 attenders, 130 individuals supplied informed consent for personal and maternal interview. Of the 200 who did not participate, 63 were ineligible because their mother was dead, non-English-speaking, or living abroad, and 200 individuals (102 males and 98 females) refused to participate. Of the 130 individuals who had given permission to contact their mother and who had completed the proband interview, 28 were not included because their mother subsequently refused. Two more were excluded because they suffered from schizophrenia.

Family History

Cases. The method used to collect information on first-degree relatives has been described elsewhere [Sham et al., 1994]. The information was obtained from four sources. First, mothers were interviewed using the Family History Research Diagnostic Criteria [FH-RDC; Andreasen et al., 1986]. Second, whenever possible (in more than 50% of relatives) relatives were personally interviewed using the Schedule for Affective Disorders and Schizophrenia [SADS-L; Endicott & Spitzer, 1978] to obtain RDC diagnoses blind to information on the proband. SADS diagnoses took precedence over FH-RDC diagnoses in case of diagnostic disagreement. A separate investigation has established that the Family History Method has rather low sensitivity but high specificity in relation to the SADS-L [Davies et al., 1997]. The other two sources of informa-

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RELATIONSHIP MODEL		FH(E+)/FH(E-)	
Type of correlation	Type of interaction	Cases	Controls
1. Zero: 	1. None: 		
	2. Additive: 		
	3. Multiplicative: 		
2. Positive: 	1. None: 		
	2. Additive: 		
	3. Multiplicative: 		
3. Negative: 	1. None: 		
	2. Additive: 		
	3. Multiplicative: 		

Fig. 1. The relationship models are based on the different possible combinations of correlation (first column) and interaction (second column) between genes and environment. For example, in the first row is depicted absence of genotype-environment correlation (genes do not influence likelihood of exposure to the environmental risk factor), in combination with each of three possible types of interaction. In the case of additive interaction the effects of both risk factors occurring together is equal to the sum of their separate effects, whereas in the case of multiplicative interaction the effects of both risk factors alone are "phenocopies" of genetic cases. In the absence of interaction, cases of illness caused by environmental risk factors are depicted for each combination of correlation, on the one hand, and interaction, on the other. According to the information in the last two columns, a positive association between family history and environmental exposure in both cases and controls is always indicative of positive correlation between genes and environment. Abbreviations: G = genes; E = environment; R(G) = genetic risk in absence of environmental risk factor; F(E) = environmental risk in absence of genetic risk; R(G+E) = combined risk of genes and environment; FH(E+)/FH(E-) = rate of familial morbid risk among individuals exposed or not exposed to the environmental factor.

tion consisted of medical records of the relatives with a psychiatric diagnosis and probands' case notes.

Controls. In controls, information on relatives was obtained through maternal interview using the FH-RDC. Information on family history with the FH-RDC was collected through personal interview with the mother for both cases and controls. Therefore, psychiatric history of the mother was assessed in a similar fashion in cases and controls.

Obstetric Complications

Mothers were interviewed with regard to obstetric history by a rater who remained blind to the case diagnosis. OCs were rated as absent or definitely present according to the scale of Lewis et al. [1989], except that short labour (less than 3 hours) was not considered as a definite complication. The scale has been validated by O'Callaghan et al. [1990]. At least one definite complication on this scale was required for allocation to the group who had experienced OCs; absence of such occurrences, or the presence only of "equivocal" signs, resulted in allocation to the group who had not experienced OCs. Information on 16 items (individual OCs) was obtained for each proband (Table I).

Analysis

Cases. Relatives' diagnoses were grouped into three categories: 1) schizophrenia; 2) affective disorders, using both a narrow "severe" definition of affective disorder (owing to the common and nonspecific nature of broadly defined depression) by including only relatives with a diagnosis of bipolar disorder or with a diagnosis of major depression requiring psychiatric admission, and a group with "mild" illness (other depressive disorders); and 3) psychosis (schizophrenia, schizoaffective disorder, delusional disorder, unspecified psychosis).

Controls. In relatives of the control group, only affective disorders (depression) were sufficiently prevalent to include in the analyses and the severe-mild distinction could not be made.

Statistical Analysis

Tests for associations between OCs in the proband and mental illness in the relatives were performed using Cox proportional hazard regression. Associations were expressed as the hazard ratio (HR). The HR is the exponentiated regression coefficient of the Cox propor-

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Table I. Obstetric Complications in Cases/Controls and Familial Morbid Risk of Psychiatric Disorder

Proband obstetric variables	Hazard ratio from Cox regression (95% CI: adjusted for age and sex of relatives) ¹			
	Family history			Controls Unipolar depression (all relatives)
	Cases			
	All psychoses (all relatives)	Schizophrenia (all relatives)	All affective disorders (all relatives)	
All OCs	1.3 (0.7-2.3)	0.6 (0.2-2.0)	1.9 (1.2-3.1) ³	1.5 (0.6-3.7)
Hyperemesis gravidarum	0.9 (0.1-6.4)	2.9 (0.4-22.0)	2.1 (0.6-6.9)	**
Low or normal forceps	1.2 (0.4-4.0)	2.2 (0.5-9.8)	0.9 (0.3-2.7)	2.0 (0.5-8.5)
High or difficult forceps	0.8 (0.2-3.3)	1.3 (0.2-9.7)	1.2 (0.4-3.3)	*
Pre-eclampsia	2.0 (0.8-5.0)	0.9 (0.1-7.0)	2.9 (1.4-6.0) ³	2.4 (0.8-7.3)
Elective cesarean	*	*	*	2.0 (0.5-8.5)
Non-elective cesarean	2.1 (0.5-8.6)	2.9 (0.4-21.8)	1.4 (0.4-2.9)	5.9 (1.7-20.7) ³
Weeks in incubator	0.6 (0.1-4.1)	*	*	3.4 (0.8-14.9)
Premature ruptured membrane	*	*	1.5 (0.2-10.7)	**
Infection during pregnancy	*	*	0.6 (0.2-2.5)	1.3 (0.8-2.1)
Antepartum hemorrhage or threatened abortion	1.4 (0.30-5.7)	1.7 (0.2-12.8)	1.5 (0.5-4.8)	*
Pregnancy duration				
Normal (37-42 wk)	1	1	1	1
Premature (<36 wk)	1.4 (0.3-6.0)	1.8 (0.2-14.0)	0.6 (0.1-4.3)	1.1 (0.1-8.2)
Post-mature (>42 wk)	0.5 (0.2-1.7)	0.5 (0.1-3.7)	1.2 (0.6-2.5)	2.2 (0.9-5.6)
Birth weight in grams (<2,500 g)	0.4 (0.1-1.8)	0.6 (0.1-4.6)	1.0 (0.4-2.4)	1.3 (0.3-5.4)
Twin	1.3 (0.3-5.4)	1.6 (0.2-12.3)	2.1 (0.8-5.7)	2.2 (0.5-9.6)
Presenting part: breech vs. other	0.6 (0.1-4.5)	*	2.8 (1.2-6.4) ²	5.1 (1.5-17.6) ²
Mother's age at delivery (>35 yr)	0.2 (0.0-1.1)	*	0.4 (0.1-1.1)	0.4 (0.1-1.7)
Problems in first postnatal months				
Poor feeder	*	*	2.9 (0.4-21.2)	**
Blue baby	1.7 (0.2-12.4)	*	*	
Infection	2.2 (0.3-16.4)	*	*	
Other	*	*	*	
Labour length (>25 hrs)	0.7 (0.3-1.6)	0.5 (0.1-2.2)	1.0 (0.5-1.8)	1.5 (0.5-4.5)
Cord prolapse	*	*	*	8.9 (1.2-68.6) ³

¹Hazard ratio = 1 indicates no association between obstetric variable and family history. HR >1 indicates positive association, HR <1 indicates negative association.

²P < 0.05.

³P < 0.01.

*Cox regression did not converge.

**Data not available.

tional hazard regression procedure (STATA, 1995). It is equivalent to the relative risk of the specified psychiatric disorder in the relatives of probands with and without OCs. Thus, a unity HR indicates no association between the obstetric variable and family history (FH is equally common in individuals exposed and nonexposed to OCs). An HR greater than unity indicates a positive association (FH is more common in exposed than in nonexposed individuals). Finally, an HR less than unity indicates a negative association (FH is less common in exposed compared to nonexposed individuals). After testing for associations between family history of psychiatric disorder and overall ratings of OCs, we repeated the analyses with the 16 individual OCs. In order to examine whether any effects of OCs on familial morbid risk were specific for the mother as opposed to other relatives, tests were also conducted separately for the group of mothers and for the group of all relatives with the exception of the mother.

Possible confounders (sex of proband and relative and proband ethnicity) were entered in the regression analysis. Tests for interaction between proband diagnosis (divided into schizophrenia and other psychosis, to increase the power of the interaction term), FH and OCs were performed by the likelihood ratio test (LRS).

RESULTS

Cases

There were 151 patients (103 males; 68%) with complete maternal interview data on OCs and family history, of whom 82 (64 males) had an RDC diagnosis of schizophrenia, 26 (15 males) of schizo-affective disorder, 24 (13 males) of mania or bipolar disorder, 9 (5 males) of major depression and 10 (6 males) of unspecified functional psychosis. Forty-five probands (30%) had a history of OCs.

Relatives of Cases

Information on 600 relatives (450 males; 75%) of the patients was available, among whom 17 (2.8%) had a diagnosis of schizophrenia, 11 (1.8%) of schizo-affective disorder, 73 (12%) of affective disorder (mild: n = 52; severe: n = 21), and 4 (0.7%) of unspecified functional psychosis.

Analysis in the Cases

There was no significant association between OCs in the cases and FH of either psychosis (HR = 1.3, 95% CI: 0.7-2.3) or schizophrenia (HR = 0.6, 95% CI: 0.2-2.0 [Table I]). A significant positive association between OCs and familial morbid risk of affective disorder in the relatives (HR = 1.9, 95% CI: 1.2-3.1; P =

0.007) was found. The association held for both severe (HR = 2.7, 95% CI: 1.1–6.7; $P = 0.03$) and mild affective disorder (HR = 2.0, 95% CI: 1.1–3.5; $P = 0.02$).

For individual obstetric complications, a significant increase in the risk of affective disorder in the relatives was found for relatives of cases with a history of pre-eclampsia (HR = 2.9, 95% CI: 1.4–6.0; $P = 0.003$) and abnormal presentation (breech: HR = 2.8, 95% CI: 1.2–6.4; $P = 0.02$). With regard to pre-eclampsia, the association held for both severe (HR = 4.0, 95% CI: 1.1–14.2; $P = 0.03$) and non-severe (HR = 3.0, 95% CI: 1.2–7.1; $P = 0.01$) affective illness. Breech delivery was strongly associated with mild affective disorder (HR = 3.6, 95% CI: 1.4–9.1; $P = 0.007$), and to a lesser extent with severe disorder (HR = 1.7, 95% CI: 0.2–13.0; $P = 0.6$).

Of the 73 cases of affective disorder in the relatives, 34 were mothers to probands. Stronger effects were observed when family history in the relatives was confined to history of affective illness in the mother (HR pre-eclampsia = 4.4, 95% CI: 1.5–13.1; $P = 0.009$; HR breech = 4.2, 95% CI: 1.5–12.2; $P = 0.008$) (Table II).

In the association between family history and all OCs, there was no evidence for interaction with proband diagnosis (all psychoses: LRS = 0.4, $P = 0.2$; affective disorder: LRS = 0.4, $P = 0.1$; the model containing schizophrenia interaction terms would not converge due to insufficient data points).

Obstetric Complications and Familial Risk

Controls

The sample of controls consisted of 100 healthy individuals (66 males; 66%). Twenty-eight probands (28%) had a history of OCs.

Relatives of Controls

Information was collected on 461 first-degree relatives of control subjects (231 males; 50%). Twenty-three relatives (5%) had a diagnosis of unipolar depression. No other diagnoses were sufficiently prevalent to be included in the analyses.

Analysis in the Controls

In the controls, FH of affective disorder was positively, though not significantly, associated with overall ratings of OCs (HR = 1.5, 95% CI: 0.6–3.7; $P = 0.4$). Strong associations were apparent with individual complications, such as breech presentation (HR = 5.1, 95% CI: 1.5–17.6; $P = 0.009$), non-elective cesarean (HR = 5.9, 95% CI: 1.7–20.7; $P = 0.005$) and cord prolapse (HR = 8.9, 95% CI: 1.2–68.8; $P = 0.04$).

Of the 23 relatives with a diagnosis of unipolar depression, 14 were mothers to probands. As in the cases, an increase in the number and strength of the effects could be observed when family history was confined to affective illness in the mother (breech presentation: HR = 7.0, 95% CI: 1.5–33.4; $P = 0.01$; pre-eclampsia: HR = 4.4, 95% CI: 1.1–17.0; $P = 0.03$; low forceps: HR = 5.2, 95% CI: 1.5–17.9; $P = 0.009$; non-elective cesar-

Table II. Obstetric Complications in Cases and Controls in Relation to Morbid Risk of Affective Disorder in Mothers

Proband obstetric variables	Hazard ratio from Cox regression (95% adjusted for age and sex of relatives) ¹			
	Family history			
	Cases		Controls	
	All affective disorders (all relatives except mother)	All affective disorders (mother)	Unipolar depression (all relatives except mother)	Unipolar depression (mother)
All OCs	2.4 (1.2–4.6) ²	2.1 (1.0–4.2) ²	0.9 (0.2–4.3)	2.2 (0.7–6.6)
Low or normal forceps	0.8 (0.1–5.8)	1.3 (0.3–5.5)	*	5.2 (1.5–17.9) ³
High or difficult forceps	2.3 (0.7–7.5)	0.6 (0.1–4.8)	*	*
Pre-eclampsia	3.1 (1.2–8.2) ²	4.4 (1.5–13.1) ³	1.2 (0.1–9.3)	4.4 (1.1–17.0) ²
Elective cesarean	*	*	2.6 (0.3–10.5)	1.5 (0.2–11.9)
Non-elective cesarean	*	4.0 (0.9–17.0)	*	28.9 (6.6–127.3) ³
Weeks in incubator	*	*	*	7.5 (1.6–35.5) ⁴
Infection during pregnancy	1.1 (0.2–8.2)	0.5 (0.1–3.8)	1.5 (0.8–3.1)	1.1 (0.6–2.2)
Antepartum haemorrhage or threatened abortion	1.7 (0.4–7.2)	1.6 (0.2–12.2)	*	*
Pregnancy duration				
Normal (37–42 wk)	1	1	1	1
Premature (<36 wk)	1.3 (0.2–9.9)	*	*	1.5 (0.2–12.2)
Post-mature (>42 wk)	1.7 (0.7–4.6)	1.1 (0.4–3.2)	1.8 (0.4–8.5)	2.6 (0.8–8.9)
Birth weight in grams				
<2,500 g	0.5 (0.1–2.2)	1.9 (0.7–5.6)	1.7 (0.2–13.6)	0.9 (0.1–7.1)
Twins	0.6 (0.1–4.3)	5.6 (1.7–18.7) ³	2.2 (0.3–18.0)	2.3 (0.3–17.9)
Presenting part: breech vs. other	2.4 (0.6–10.1)	4.2 (1.5–12.2) ³	3.2 (0.4–25.8)	7.0 (1.5–33.4) ²
Mother's age at delivery (>35 yr)	0.2 (0.0–1.2)	0.6 (0.1–2.7)	0.7 (0.1–5.7)	0.2 (0.0–2.0)
Labour length (>25 hrs)	0.7 (0.3–1.6)	1.4 (0.6–3.2)	2.9 (0.6–14.0)	1.0 (0.2–4.4)
Cord prolapse	*	*	*	43.7 (4.0–482.6) ³

¹Hazard ratio = 1 indicates no association between obstetric variable and family history. HR >1 indicates positive association. HR <1 indicates negative association.

² $P < 0.05$.

³ $P < 0.01$.

*Cox regression did not converge.

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ean: HR = 28.9, 95% CI: 6.6–127.3; $P < 0.001$; cord prolapse: HR = 43.7, 95% CI: 4.0–482.6; $P = 0.002$; weeks in an incubator: HR = 7.5, 95% CI: 1.6–35.5; $P = 0.01$, [Table II].

Both in the cases and the controls, results were generally in the direction of a positive association. The effects of the individual complications were independent of each other: their effects remained significant when adjusted for each other.

Adjustment for sex and ethnicity of the proband only changed the parameters by a very small amount and did not change the pattern of the results, with the exception of the association between pre-eclampsia and family history of affective disorder in the cases, where adjustment for ethnicity reduced the strength of the association. However, the reduced effect remained highly significant nonetheless (all relatives: HR = 1.4, 95% CI: 1.2–1.8; $P < 0.01$; mother only: HR = 1.7; 95% CI: 1.2–2.3; $P < 0.01$).

Age at Delivery and Age of Onset of Affective Disorder

In those mothers of cases who had a history of affective disorder, this disorder preceded delivery in 34% of cases and only three mothers had had onset of affective disorder within three years after delivery. In the mothers of controls, these figures were 22% and 2, respectively. Exclusion of mothers who had onset of affective disorder within three years of an OC-positive pregnancy or delivery only changed the parameters by a very small amount.

DISCUSSION Methodological Issues

The obstetric data in this study were assessed retrospectively by gathering information from interviews with the probands' mothers. Although this source of information may be subject to information bias, work by O'Callaghan et al. [1990] suggests this is slight. Furthermore, information with regard to obstetric history was assessed blind to family history. The relatively large sample size reduced the risk of type II error. However, tests for interactions have low statistical power and thus we cannot definitely exclude the possibility that the relationship between OCs in the proband and family history of mental illness is specific for any subtype of psychotic disorder in the proband. Similarly, the results with individual OCs should be viewed with caution, as the prevalence of some OCs was low, yielding large effect sizes with very wide confidence intervals in some instances (e.g., cord prolapse). It is important to note, however, that results on two individual OCs (pre-eclampsia and breech presentation) were independently replicated in both cases and controls. It is unlikely that our results represent chance findings, given that the number of significant findings (18), in combination with the strength of statistical associations, was well above chance expectation (8).

Family history was not assessed in a similar way in cases and controls, control relatives (other than the mother) with affective disorder having a greater probability of being misclassified because personal interviews were not carried out in relatives of controls. How-

ever, associations were most marked with psychiatric disorder in the mother, and mothers of both cases and controls were personally interviewed with the same instrument, ruling out such bias. Because mothers were personally interviewed, diagnostic sensitivity would have been higher in this group, which is reflected in the higher prevalence of depressive disorder in probands' mothers relative to other family members. Furthermore, the differences in the assessment of family history would only have biased the results if cases of affective disorder in the relatives of controls had been missed as a function of the proband having had an obstetric complication. This is unlikely, as any bias would have been more likely to operate the other way round (see below).

One obvious explanation for the findings is that mothers with a history of depressive disorder are more likely to report negative events in general. However, this cannot explain why the associations between maternal morbid risk of depression and individual OCs were stronger in controls. Furthermore, if a negative recall bias had been operating, one might have expected to see differences between mild and severe affective disorders, in that having suffered with severe depression might make one more prone to differential recall. Finally, prospective studies have ascertained a genuine association between maternal depressive symptoms and risk of poor pregnancy outcomes [Hedegaard et al., 1993; Orr & Arden Miller, 1995].

Findings

OCs in both psychotic and well control probands were found to be positively associated with affective illness in the relatives, although association with OCs as defined by the Lewis and Murray scale failed to reach statistical significance in the controls. However, the rate of depression in relatives of controls was low because of the low sensitivity of the FH-RDC for depression. Analyses confined to probands' mothers (who were personally interviewed regardless of proband case/control status) yielded similar effect sizes for OCs as defined by the Lewis and Murray scale in both cases and controls, and even though the group of control relatives was smaller, associations with individual OCs were more frequent and of larger effect size in the control group.

Familial Morbid Risk of Psychotic Disorder. The lack of significant associations between OCs and FH of psychosis and schizophrenia is not compatible with positive correlation between these two risk factors (Fig. 1). The absence of correlation agrees with the conclusion by McNeil [1991] that it is unlikely that OCs are the expression of genes predisposing to schizophrenia.

Family History of Affective Disorder. From Figure 1 it can be derived that positive associations between OCs and family history of depression in both cases and controls exclude any model of interaction in the absence of correlation or with negative correlation. Several other explanations remain. These include positive correlation in either the presence or absence of interaction.

Positive correlation can be envisaged in two ways. First, an affective diathesis in the fetus may increase the risk for adversity during pregnancy and delivery. Liston and Kilpatrick [1991] invoked the possibility of

fetal genes contributing to the development of pre-eclampsia, whereas atypical presentations may similarly be the result of, possibly genetically determined, fetal abnormalities [Günther-Genta et al., 1994]. This possibility, however, is unlikely in view of the fact that the effect of familial morbid risk on OCs was confined, among the relatives, to the *mothers* of probands.

A second, more likely, explanation for the correlation between family history of depression and OCs is that mothers with a predisposition for affective illness are at heightened risk of experiencing (and therefore their child as well) certain complications. Indirect evidence for this supposition comes from the following observations: 1) as mentioned above, prospective studies have ascertained a genuine association between maternal depressive symptoms and risk of poor pregnancy outcomes [Hedegaard et al., 1993; Orr & Arden Miller, 1995]; and 2) mothers about to give birth to children destined to develop psychosis more often report affective disturbance. Support for the latter observation comes from two recent cohort studies. First, Sacker and colleagues [1995] showed that mothers of babies destined to become schizophrenic were not only more likely to have experienced obstetric complications, but were also 11 times more likely to have suffered psychological problems than control mothers, and 2.5 times more likely to have had poor antenatal attendance. Second, a recent Finnish birth-cohort study by Jones and colleagues (submitted), reported that 22.4% of mothers pregnant with children who would develop schizophrenia as adults not only experienced more obstetric complications, but also rated themselves as depressed between 24 and 28 weeks of pregnancy, compared to only 13.7% of control subjects, a significant difference. In addition, more indirect support comes from a study by Guth and colleagues [1993], who showed that early-onset affective disorder with high familial loading was also associated with OCs in a patient sample.

The mechanism between maternal depressive symptoms and adverse prenatal and/or perinatal outcomes may be direct or indirect. For example, altered compliance with prenatal care may result in a higher frequency of pre-eclampsia or breech presentation [Rayl et al., 1996]. Other (behavioral) mechanisms leading indirectly to associations between depressive mothers and pregnancy outcome may be drug use, poor weight gain, smoking, and alcohol-use habits. Orr and Arden Miller [1995] also proposed more direct mechanisms, such as altered hormonal/biochemical response due to depressed mood.

Reported high rates of depression in the mother may be the result of living with a psychotic patient, who also happens to have suffered more obstetric complications. This is unlikely, because in the controls, who were well, a similar association existed. Alternatively, the complicated pregnancy/delivery itself may have brought about depression in the mothers of cases and controls. This is also unlikely, given that many affective episodes preceded delivery, and the fact that exclusion of affective episodes in the three years after an OC-positive proband birth did not change the results.

Practical Significance. If the likelihood of exposure to OCs is influenced by the factors that contribute

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to the familial aggregation of depressive symptoms in psychotic patients, the question arises what proportion of the total number of OCs can be directly attributed to these factors (the population attributable fraction; PAF). Given a relative risk of 1.9 (the HR for OCs and familial morbid risk of affective disorder), and a familial morbid risk for depression of around 12% in the psychotic probands, the PAF can be calculated [Hennekens and Buring, 1987] approximately as $[(1.9 - 1) / 1.9] \times 0.12 = 5.6\%$. Therefore, although significant associations exist, they may not be of great practical significance and can explain only a small part of the reported excess of OCs in patients with schizophrenia.

Another question to consider is whether the OCs which showed the strongest associations with familial morbid risk for affective disorder are also the OCs that have been shown to carry the highest relative risk for schizophrenia and related psychotic disorders. A recent meta-analysis carried out by Geddes and colleagues [1997] on prospective and retrospective studies using the Lewis-Murray scale failed to show significant associations between OCs, such as breech presentation and pre-eclampsia, and later risk for schizophrenia, whereas these two OCs carried the highest relative risks in both psychosis cases and controls in the present study. However, of the individual OCs that were found to predict later schizophrenia in the meta-analysis by Geddes and colleagues [1997], fetal hypoxia was the potential common mechanism. As pre-eclampsia also induces fetal hypoxia and a recent prospective study by Kendell and colleagues [1996], with the largest sample of cases to date, reported very high relative risks for pre-eclampsia, at least one of the associations between OCs and familial morbid risk reported in this study may be of aetiological importance. Thus, pre-eclampsia may mediate some of the relationship between family history of affective disorder and psychosis.

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APPENDIX. Genotype-environment interaction/correlation and rate of illness in relatives of probands exposed to the predisposing environment

The mathematical considerations below apply to any two risk factors. In the present paper, E1 represents obstetric complications and E2 represents family history of psychiatric illness. By examining the effect of the presence or absence of obstetric complications on the rate of psychiatric illness in family members in patients and controls, we may be able to infer the likely patterns of interaction and correlation between obstetric complications and family history.

The interaction between two causes is specified by the pattern of risk in four cells. If B is the baseline risk, and R is the increased risk associated with either of the risk factors E1 and E2 (assumed to have equal effect size R for convenience), then the patterns of interaction among cases (disease positive, D+) can be written as:

	E ₂ ⁻	E ₂ ⁺
E ₁ ⁻	B	B+R
E ₁ ⁺	B+R	B+R+I

where I is an interaction term, defined as I=0 for no interaction, I=R for additive interaction, and I=R²/B for multiplicative interaction. Among controls (D-), the pattern of interactions are then

	E ₂ ⁻	E ₂ ⁺
E ₁ ⁻	1-B	1-(B+R)
E ₁ ⁺	1-(B+R)	1-(B+R+I)

The correlation between two causes is specified by the pattern of joint occurrence of the two factors. If F is the frequency of the two risk factors (assumed to be equal for simplicity), then the three distinct patterns of correlation are

	E ₂ ⁻	E ₂ ⁺	
E ₁ ⁻	(1-F)(1-F)+C	(1-F)F-C	1-F
E ₁ ⁺	(1-F)F-C	FF+C	F
	1-F	F	

so that zero correlation is defined by C=0, negative correlation is defined by C<0, and positive correlation is defined by C>0. The joint frequency distribution of the two risk factors and the disease in the population is then

E ₁	E ₂	D+	D-
-	-	B[(1-F)(1-F)+C]	(1-B)(1-F)(1-F)+C
-	+	(B+R)[(1-F)F-C]	1-(B+R)[(1-F)F-C]
+	-	(B+R)[(1-F)F-C]	1-(B+R)[(1-F)F-C]
+	+	(B+R+I)(FF+C)	1-(B+R+I)(FF+C)

We wish to examine the consequences of different levels of interaction and correlation between the two risk factors, on the rate of E2 among individuals exposed or not exposed to E1, in patients and in controls. The consequences of three levels of interaction (I=0, R, R+RR/B) and three levels of correlation (C<0, C=0, C>0), in terms of the ratios P(E2+|E1+)/P(E2-|E1-) for cases, P(E2+|E1+)/P(E2-|E1-) for controls, are as follows:

Correlation	Interaction					
	I=0		I=R		I=R+RR/B	
	D+	D-	D+	D-	D+	D-
C<0	<1	<1	<1	<1	<1	<1
C=0	<1	>1	<1	<1	=1	<1
C>0	<1	>1	<1	<1	>1	<1

Metabolic stress as a paradigm to elucidate genotype-environment interaction in psychosis

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ABSTRACT

Background. Previous work suggests that individuals with schizophrenia display an altered HVA-response to metabolic stress (Breier et al 1993). The present study replicated and extended this paradigm, including individuals with elevated genetic risk for schizophrenia.

Methods. Patients with psychosis (n=50), non-psychotic first-degree relatives of patients with psychosis (n=51) and controls without psychosis (n=50) underwent, in randomised order, double-blind administration of placebo and the glucose analog 2-deoxy-D-glucose (2DG), which induces a mild, transient clinical state of hypoglycaemia. Plasma HVA and cortisol were assessed twice before the start of the 2DG/placebo infusion (baseline values), as well as four times post infusion. Data were analysed using multi-level random regression techniques.

Results. During the stress condition, a significant increase in plasma HVA (condition x time interaction: $LRS=167.78$, $p<0.001$) and cortisol (condition x time interaction: $LRS=652.63$, $p<0.001$) was found. The increase in plasma HVA level during the stress condition differed significantly between the groups ($LRS=6.54$, $p=0.01$), the strongest increase being present in patients, followed by first the relatives and finally the controls. Results for cortisol revealed a suggestive group x condition x time interaction ($LRS=3.54$, $p=0.06$) in the opposite direction, indicating that the strongest increase in cortisol was seen in controls, followed by first the group of relatives and finally the patients.

Conclusion. Patients with psychosis and their first-degree relatives may have an altered neurobiological response (increased plasma HVA levels in the presence of diminished plasma cortisol levels) to metabolic stress, suggesting a model of genetic transmission of sensitivity to environmental stresses or genotype-environment interaction.

INTRODUCTION

Individuals with schizophrenia have been found to have an increased HVA-response to metabolic stress as compared to healthy controls (Breier et al 1993), suggesting that this group of patients may have an abnormal regulation of dopaminergic (DA) and/or noradrenergic (NA) systems during stress conditions. In addition, the DA/NA mediated response to stress may be dissociated from the hypothalamic-pituitary-adrenal response under similar conditions (Breier et al 1993). Metabolic stress can be induced by intravenous infusion of the glucose analog 2DG. This glucose analog inhibits intracellular glucose metabolism and produces a mild, transient clinical state of hypoglycaemia (Welle et al 1980). The effect of this stressor on catecholamine and neuroendocrine systems, can be assessed by repeatedly measuring homovanillic acid (HVA) and cortisol in plasma over time (Breier 1989; Breier et al 1991; Breier et al 1993; Breier et al 1988b).

If patients with schizophrenia have altered stress-sensitivity, it is attractive to hypothesize that part of the sensitivity to environmental stress is transmitted genetically, as implied by the stress-vulnerability model (van Os et al 1998; Zubin et al 1977). First-degree relatives share, on average, 50% of their genes with their patient relative, and have repeatedly been found to have intermediary values for, for example, cognitive abnormalities (Cannon et al 1994; Krabbendam et al 2001) and cerebral structural abnormalities (Cannon et al 1998; Sharma et al 1997). Given the fact that the shared environment has no substantial influence on familial resemblance in studies of schizophrenia liability (Cardno et al 1999), being a first degree relative can be considered a useful proxy measure of genetic risk in studies on schizophrenia. We thus predicted that i) patients would have an abnormal metabolic stress response, and ii) that first degree relatives of patients would have values intermediary to those of patients and controls. The present study investigated this issue by applying the 2DG paradigm (Breier et al 1993) to patients with psychotic illness, non-psychotic first-degree relatives and healthy controls.

METHODS

Subjects

This study is part of the Maastricht Psychosis Study (Krabbendam et al 2001). Patients with a life-time history of psychosis (of at least two weeks) according to the RDC criteria (Spitzer et al 1978), were recruited at the community mental health centre in Maastricht, the Netherlands. All patients were in remission or in partial remission defined as not in need of hospital admission or intensive case management. Non-psychotic first-degree relatives were recruited through the participating patients, as well as through local relatives associations. Relatives were free from a lifetime history of psychosis. Unrelated healthy controls were sampled from the general population, using a mailing procedure to randomly selected households in the local catchment area. Controls were excluded whenever they

had a personal or family history of psychosis or other major psychiatric disorder requiring hospital admission.

Other inclusion criteria for all participants (patients, relatives and controls) were: aged 18-55 years, and being in good health as determined by a physical examination, electrocardiography and routine laboratory investigations. Individuals with neurological, endocrine, cardiovascular and/or other serious medical disorders were excluded, as well as individuals with a history of severe head trauma with loss of consciousness. In addition, individuals who used alcohol in excess of five standard units per day were also excluded, as well as patients who used illicit drugs on a weekly basis.

Clinical and diagnostic procedures

Patients (n=50), relatives (n=51) and controls (n=50) were interviewed with the expanded version of the Brief Psychiatric Rating Scale (BPRS; (Lukoff et al 1986), the PANNS (Kay et al 1987; Kay et al 1988), and case note and other historical material was additionally screened for symptoms listed in the OCCPI (McGuffin et al 1991). Where necessary, additional information was derived from interviews with the responsible medical officer. Based on the combined information, the computerised program OPCRIT (McGuffin et al 1991) yielded RDC-diagnoses. There were 40 patients (80%) with a diagnosis of schizophrenia, and 10 patients received a diagnosis of schizo-affective disorder (20%). In addition, six relatives were diagnosed with major depression.

Groups were frequency matched on age, sex and educational level (see Table 1). Of the 50 patients, 47 were on antipsychotic medication. The mean age of first psychotic symptoms was 22.0 (5.7). The mean total score of the patients on the BPRS was 39.1 (SD=10.3).

Table 1. *Demographic characteristics of study participants*

	Patients (sd) n=50	Relatives (sd) n=51	Controls (sd) n=50	F Statistic (df)	P
Gender ratio: Male/Female	26/24	26/25	25/25		
Age, yrs	31.2 (7.5)	37.2 (11.3)	35.0 (8.9)	5.3 (2, 148)	.01
Educational achievement	3.6 (1.4)	4.6 (1.7)	4.4 (1.6)	5.64 (2, 148)	.004
BPRS	39.1 (10.3)	28.4 (5.2)	25.6 (2.3)	54.8 (2, 146)	.001

2-Deoxy-Glucose protocol

All subjects underwent double-blind administration of the glucose analog 2DG and placebo, in randomised order. The 2DG doses were 50 mg/kg mixed in 100 ml of isotonic saline. Placebo was a comparable volume of isotonic saline (NaCl, 0.9%). Both conditions (2DG/placebo) were given within one week, with at least two days in between. Subjects had to fast from midnight prior to both test days, and were only allowed to drink water *ad*

lib. In addition, they were not allowed to take alcohol in excess of 2 standard units the day before the test, and subjects restricted cigarette smoking on the morning of the test day to not more than 2 cigarettes, and not within 1.5 hour before the start of the test. During the test, subjects were lying on a bed and remained resting there from 8.45 am until 12.30 pm. At 8.45, an intravenous catheter was inserted in the antecubital fossa and kept patent with a slow drip of isotonic saline. At 9.50 hrs, two baseline venous blood samples were taken 10 minutes (mins) and 0 mins prior to infusion. At 0 mins, 2DG or placebo was infused over a period of 20 mins. Four more blood samples were taken at 60 mins, 90 mins, 120 mins and 150 mins after infusion.

In order to test the validity of the metabolic stress paradigm, we measured the effect of 2DG on the physiologic variables blood pressure, pulse and temperature at fixed intervals. In addition, patients were asked to fill in questionnaires about their physical state, such as level of tiredness, trembling, feeling warm or cold (subjective experience list, 5-point scales) as well as about subjective experiences of the tension and tiredness items (5-point scales) of the Profile of Mood States (Wald et al 1990).

Blood was collected in tubes containing 0,5 ml of an EDTA (40mg/ml) and $\text{Na}_2\text{S}_2\text{O}_5$ (20 mg/ml) solution, gently mixed and immediately placed on ice. Subsequently, plasma was obtained by centrifugation (15 mins at 3000 r/min) in a refrigerated centrifuge (5° C) and immediately stored at -80° C until assaying.

A 515 WATERS isocratic HPLC was used for assaying HVA and MHPG, with a Symmetryshield RP18 25 cm column for the separation of the compounds. The mobile phase was an acetate 20 mM/methanol (98:2) buffer at pH 5,75. Samples were prepared online with an ASTED XL (Gilson), and the detection was performed with a Coulochem II (ESA) coulometric detector, equipped with a 5020 guard cell set at 200 mV and a 5011 analytical cell (E1 : +150 mV ; E2 : +450 mV ; sensitivity: 20 nA). Quantification was based on the ratio between area of the compound and area of the internal standard. The retention times of 4-hydroxy-3-methoxyphenylglycol (MHPG) and homovanillic acid (HVA) were respectively 23,5 and 26,6 minutes at a flow of 0,7 ml/minute. The detection of quantification (sensitivity) was 0,5 ng/ml for both compounds. Intra-assay variability was 3% for MHPG and 5% for HVA. Inter-assay variability was 7% for MHPG and 9% for HVA.

Cortisol levels were determined from plasma by radioimmunoassay with an intra-assay and interassay variability of 8% and 10%, respectively.

All subjects gave written informed consent conform the local ethics committee guidelines.

Statistical analyses

The data were analysed with the STATA computer program (version 6)(STATA 1999). A three-level group variable was constructed reflecting risk for schizophrenia with controls as the baseline, relatives in the middle, and patients in the highest category. The condition variable reflected two levels, the placebo condition (reference), and the 2DG condition. HVA and cortisol were sampled on six occasions (time 1-6). This time variable (1-6) was broken down into two variables (timeA and timeB) reflecting the two pre- and the four post infusion measurement occasions. TimeB served as the variable of interest, timeA as covariate. The mean HVA/cortisol level of the two pre-infusion samples for each person and each condition were used to construct a baseline HVA variable (HVA_base) and baseline cortisol variable (CORT_base).

The association between metabolic stress on the one hand, and plasma HVA and cortisol (the continuous outcome variables) on the other, was investigated. Group, condition and timeB, as well as the two and three-way interactions between these variables were used as the explanatory variables. The baseline variables (HVA_base in HVA models, and CORT_base in cortisol models), as well as the timeA variable were used as covariates in all models. In addition, age, sex, cigarette smoking/non-smoking and mean alcohol intake per week were used as covariates in separate analyses to adjust for their *a priori* hypothesized confounding effects. Interaction terms were evaluated by Likelihood Ratio tests (Clayton et al 1993). As the average measure of HVA or cortisol is assumed to vary across persons, two observations will be more similar if they are from the same person. Our design of repeated measures within the same person therefore compromised statistical independence of the observations. In order to deal with this issue, multilevel random regression models were fitted (Goldstein 1987). In multilevel regression, dependency of observations within persons is taken into account by estimating a within-person as well as a between-person level variance. Thus, the model used had two levels: measurement occasions (level 1) clustered within subjects (level 2). Effect sizes of explanatory variables were expressed as regression coefficients (β) from the multilevel models.

The regression coefficients from the multilevel models were used to calculate predicted HVA and cortisol levels in order to plot them and examine the nature of the three- and two-way interactions. For example, if the regression coefficients from the HVA multilevel model, were: group x condition x timeB interaction: $\beta=0.0451858$; condition x timeB interaction: $\beta=0.8757972$; condition: $\beta=-0.0306707$; timeB: $\beta=-1.357254$; timeA: $\beta=0.2779545$; group: $\beta=-0.1104093$; HVAbase: $\beta=0.848990$; constant: $\beta=1.043817$, the predicted HVA in the three groups was calculated using the formula:

$$\text{Predicted HVA} = 1.043817 + 0.0451858 \cdot (\text{group} \times \text{condition} \times \text{timeB}) + 0.8757972 \cdot (\text{condition} \times \text{timeB}) + -0.0306707 \cdot (\text{condition}) + -1.357254 \cdot (\text{timeB}) + 0.2779545 \cdot (\text{timeA}) + 0.1104093 \cdot (\text{group}) + 0.848990 \cdot (\text{HVA\_base}).$$

Physical and behavioural variables

Separate analyses were conducted with physical variables (blood pressure, pulse, temperature) and subjective experience of tension and tiredness as the outcome variables and condition and time as the exposure variables.

RESULTS*Plasma HVA.*

Mean levels of HVA (ng/ml) at the six measurement points during the stress and placebo conditions are presented in Table 2. There was a significant effect of condition on HVA ($\beta = 1.51$, $p < 0.0001$). The effect of timeB on HVA in the 2DG condition was greatest in the patient group ($\beta = 0.66$, $p < 0.0001$), followed by the relatives ($\beta = 0.46$, $p < 0.0001$) and the controls ($\beta = 0.28$, $p < 0.0001$). In addition, a significant condition $\times$ time interaction was found (Likelihood Ratio Statistic = 167.78, $p < 0.001$), indicating an increase in HVA over time in the 2DG condition. When group was added to the statistical model and a three-way interaction fitted, a significant group $\times$ time $\times$ condition interaction was apparent (LRS = 6.54, $p = 0.011$). Adjustment for age, sex, alcohol intake and cigarette smoking did not change the results (group $\times$ time $\times$ condition interaction: LRS = 6.33, $p = 0.012$). In order to further inspect the nature of the three-way interaction, predicted HVA levels, derived from the model with the three-way interaction, were plotted for each group in both conditions (Fig. 1 and 2). This revealed that the strongest increase in HVA over time in the 2DG condition was seen in the patient group, with the group of relatives showing an intermediate position between patients and controls.

Table 2. *Effects of 2-deoxy-D-glucose on plasma HVA in psychotic patients, non-psychotic first-degree relatives and healthy controls.*

	HVA (ng/ml)					
	Baseline					
	Time1	Time2	Time3	Time4	Time5	Time6
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
<i>Controls (n=50)</i>						
Placebo	10.2 (7.0)	9.9 (7.0)	9.2 (6.7)	8.3 (5.6)	8.0 (5.4)	7.8 (4.9)
2DG	9.0 (3.9)	8.7 (4.2)	8.8 (4.1)	9.4 (4.3)	9.8 (4.3)	9.8 (4.7)
<i>Relatives (n=51)</i>						
Placebo	9.0 (5.8)	8.7 (5.5)	7.8 (4.8)	7.8 (4.8)	7.4 (4.3)	7.4 (4.1)
2DG	8.3 (4.0)	8.1 (3.8)	8.4 (3.1)	8.9 (3.3)	9.4 (3.5)	9.9 (3.6)
<i>Patients (n=50)</i>						
Placebo	10.1 (4.6)	9.7 (4.2)	8.4 (4.2)	8.0 (4.0)	7.6 (3.4)	7.4 (3.2)
2DG	9.5 (4.4)	9.4 (4.1)	9.0 (3.6)	9.9 (4.2)	10.5 (4.7)	11.3 (5.4)

Condition x time interaction: LRS=167.78, $p<0.001$

Condition x time x group interaction: LRS=6.54, $p=0.01$

Fig. 1 Modelled effects of 2-DG infusion on plasma HVA in patients with psychosis, relatives and controls

Fig. 2 Modelled effects of placebo infusion on plasma HVA in patients with psychosis, relatives and controls

Plasma cortisol.

Mean levels of cortisol (ng/μl) at the six measurement points during both conditions are presented in Table 3. There was a significant overall effect of condition on plasma cortisol ($\beta=10.45$, $p<0.0001$). The strongest effect of timeB on cortisol in the 2DG condition was seen in the controls ($\beta=2.20$, $p<0.0001$), followed by the relatives ($\beta=2.12$, $p<0.0001$), and the patients ($\beta=2.04$, $p<0.0001$). There was a significant condition x time interaction ($LRS=652.63$, $p<0.001$), indicating a significant increase in cortisol in the 2DG condition. This increase in cortisol appeared to differ between the groups, although the group x time x condition interaction was statistically imprecise ($LRS=3.54$, $p=0.06$). Adjustment for age, sex, alcohol intake and cigarette smoking did not affect the results ($LRS=3.25$, $p=0.07$). Predicted cortisol levels during both conditions were plotted, based on the regression coefficients derived from the model with the three-way interaction. This suggested the effect on plasma cortisol was strongest in the controls and weakest in the patients (Fig. 3 and 4).

Table 3. *Effects of 2-deoxy-D-glucose on plasma cortisol in psychotic patients, non-psychotic first-degree relatives and healthy controls.*

	Cortisol (ng/μl)					
	Baseline		Time3	Time4	Time5	Time6
	Time1	Time2				
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
<i>Controls (n=50)</i>						
Placebo	12.8 (5.4)	11.8 (5.0)	8.2 (4.0)	7.3 (4.0)	6.8 (3.3)	7.5 (4.2)
2DG	13.9 (8.2)	12.8 (7.6)	21.9 (6.9)	25.3 (6.6)	26.9 (7.6)	27.2 (9.9)
<i>Relatives (n=51)</i>						
Placebo	15.0 (12.8)	14.2 (12.9)	10.2 (8.7)	9.4 (8.9)	9.2 (8.8)	9.3 (8.6)
2DG	13.6 (5.8)	12.4 (5.6)	21.2 (5.1)	24.7 (6.1)	26.0 (7.0)	26.5 (7.9)
<i>Patients (n=50)</i>						
Placebo	12.5 (5.0)	11.6 (4.6)	9.1 (3.7)	8.7 (3.5)	8.6 (3.2)	8.9 (3.5)
2DG	12.4 (4.6)	11.2 (4.5)	19.1 (5.6)	23.0 (5.2)	24.2 (5.1)	24.3 (6.1)

Condition $\times$ time interaction: $LRS=652.63$, $p<0.001$

Condition $\times$ time $\times$ group interaction: $LRS=3.54$, $p=0.06$

Fig. 3 Modelled effects of 2-DG on plasma cortisol in patients with psychosis, relatives and controls

Fig. 4 Modelled effects of placebo infusion on plasma cortisol in patients with psychosis, relatives and controls

Physiologic and behavioural variables

There were significant positive condition x time interactions for systolic blood pressure (LRS=22.55, $p<0.001$), as well as for heart rate (LRS=27.10, $p<0.001$), indicating an increase in these variables over time in the 2DG condition. Diastolic blood pressure and temperature significantly decreased during the stress condition, as reflected by negative condition x time interactions (diastolic blood pressure: LRS=68.35, $p<0.001$; temperature: LRS=722.51, $p<0.001$).

There was a significant condition x time interaction for tiredness scores, indicating a significant increase in feelings of tiredness during the stress condition (LRS=9.95, $p=0.002$), as well as a statistically imprecise condition x time interaction for tension (LRS=2.92, $p=0.088$).

DISCUSSION

The present study investigated whether a proxy measure for schizophrenia genetic liability was associated with response to a metabolic stressor, expressed as changes in plasma HVA and cortisol levels, complemented by subjective questionnaire measures. Patients with psychosis and non-psychotic first-degree relatives were found to have an altered stress response as compared to controls, whereby relatives had a position that was intermediate to patients and controls in terms of effect size. The HVA response was significantly and positively associated with a proxy measure of genetic risk for schizophrenia (HVA increase in patients > relatives > controls) whereas the cortisol response was negatively associated with the genetic risk (cortisol increase in patients < relatives < controls), although this latter result was not significant at conventional alpha (i.e. the probability that this result was due to chance was 6% rather than 5%).

Methodological considerations.

The glucose-analog 2DG causes intracellular hypoglycaemia by competing with glucose-6-phosphate during the early stage of glycolysis, and inhibiting intracellular glucose utilization. This stress paradigm has been found to strongly affect DA metabolism and to cause substantial plasma elevations of cortisol levels, as well as significant effects on physiologic variables (blood pressure, heart rate, temperature) (Breier 1989; Breier et al 1991; Breier et al 1993; Breier et al 1988b), which could also be shown in this study, adding to the validity of the stress paradigm. Glucose deprivation has profound effects in the brain, for which glucose is the most important source of energy. A recent PET study (Elman et al 1999) showed that pharmacological doses of 2DG lead to widespread activation of cortical and subcortical blood flow in healthy volunteers.

Much of the plasma HVA originates from central and peripheral NA systems. Even under fasting conditions, around 75% of plasma HVA derives from the NA neuronal metabolism (Amin et al 1992; Kopin et al 1988a; Kopin et al 1988b). Nevertheless, plasma HVA is assumed to be a marker of central DA activity (Friedhoff et al 1997). Evidence supporting this hypothesis comes from studies showing similar correlations between *plasma* and *CSF* HVA on the one hand, and psychotic-like symptoms on the other hand in schizotypal personality disorder (Siever et al 1991; Siever et al 1993). In addition, a large body of evidence suggesting that central DA activity is reflected by plasma HVA levels comes from studies showing correlations between plasma (and CSF) HVA and the clinical response to antipsychotic medication (Bowers et al 1984; Brown et al 1997; Davila et al 1988; Pickar et al 1986; Pickar et al 1984; Pickar et al 1987; Sharma et al 1989). Other lines of research, however, suggest that the peripheral HVA levels are likely to be under central regulatory control, without specifically reflecting central DA activity. In fact, there is evidence for hypothalamic involvement (Yoshimatsu et al 1987) in the regulation of HVA outflow by the sympathetic nervous system. Taken together, it seems reasonable to assume that plasma HVA level changes during conditions of stress, whether or not directly reflecting central DA activity, are regulated by central factors. Moreover, the main goal of the present study was to investigate whether alterations in the general neurobiological stress-response during metabolic perturbation may be an indicator of genetic risk for schizophrenia (i.e. whether it is an endophenotype). From this perspective, it was not attempted to distinguish differential contributions of central and peripheral DA and NA systems.

Contrary to relatives and controls, almost all patients were medicated with an antipsychotic drug, influencing both dopamine and cortisol levels, although chronic use of antipsychotics has not been found to affect cortisol levels (Meador-Woodruff et al 1988). The fact that the patients showed a significantly stronger increase in plasma HVA during the 2DG condition as compared to controls indicates that (chronic) antipsychotic treatment does not preclude stress-induced increases in dopamine function, which is in agreement with other reports (Breier 1989; Breier et al 1993). Moreover, in the study of Breier and colleagues (Breier et al 1993), medicated and non-medicated groups were compared and no significant differences in the HVA and cortisol response to metabolic stress between these groups were found. In addition, prior studies have described a blunted cortisol response in non-medicated patients (Albus et al 1982; Breier et al 1988a). Lastly, as both relatives and controls were not medicated, the intermediate position of the relatives between patients and controls with regard to both (HVA and cortisol) stress responses cannot be explained on the basis of medication effects.

Plasma HVA concentrations are sensitive to various confounding factors, such as diet, exercise, alcohol, smoking, and diurnal variation (Amin et al 1997). Nevertheless, all these factors were controlled for in the present study at both the selection and the analysis stage. The HVA data were collected on two separate days within one week, with at least two days in between. Amin and colleagues have shown that plasma catecholamine metabolites in normal subjects have good reproducibility (Amin et al 1998).

In several of the analyses, "group" was used as a linear variable of continuous schizophrenia liability. By doing so, it was assumed that the difference between 0 (controls) and 1 (relatives), is the same as the difference between 1 and 2 (cases). While there is no formal way of assessing whether this assumption is correct, testing linear hypotheses of genetic risk using relatives of patients has nevertheless been proven a useful and statistically powerful way to examine gene-environment interactions (Cannon et al 1993), and has face validity given the above cited findings of intermediary values for relatives of patients for a range of schizophrenia endophenotypes.

Findings

The finding of increased HVA response during metabolic stress in patients with a diagnosis of schizophrenia replicates prior studies (Breier 1989; Breier et al 1993). The strength of the present study is the larger sample size as compared to earlier studies. In addition, this is the first study investigating the effects of metabolic stress on plasma levels of HVA and cortisol in non-psychotic first-degree relatives. The results indicate that an altered stress response as depicted by increased HVA levels and diminished cortisol levels is associated with a proxy measure of genetic vulnerability for schizophrenia. These findings are in line with a recent study (Amin et al 1999), in which first-degree relatives of patients with a diagnosis of schizophrenia not only showed inverse correlations between plasma HVA levels and negative symptoms and positive correlations between plasma HVA and attenuated positive symptoms, but also had lower plasma HVA levels than controls, consistent with the presence of negative symptoms in the relatives. As negative symptomatology has been related to the genetic diathesis of schizophrenia, the authors suggested that this negative symptomatology is based on an underlying dysfunctional brain DA activity, possibly representing part of the genetic vulnerability for schizophrenia.

There was a trend for blunted cortisol response to metabolic stress in patients, and to a lesser extent in relatives as compared to controls. This would indicate that an altered neuroendocrine stress response (dysfunctioning hypothalamic-pituitary-adrenal axis) may be associated with the genetic vulnerability for schizophrenia. However, the results were statistically imprecise and need to be replicated before definite conclusions can be drawn. Although we are not aware of studies reporting on this issue in relatives, there is,

nevertheless, some evidence for a diminished cortisol response in patients with a diagnosis of schizophrenia. In a study using lumbar puncture as the stressor (Breier et al 1988a), patients with a diagnosis of schizophrenia did not show an increase in plasma cortisol concentration whereas depressed patients and controls did. In addition, patients with a diagnosis of schizophrenia have been found to have a blunted cortisol response during a psychological stress task (Albus et al 1982; Jansen et al 1998). This was replicated in a subsequent study by Jansen et al (Jansen et al 2000), in which the blunted cortisol response was additionally found to be specifically related to psychological stress, and not to a physical (exercise) stress task. Despite the above described evidence for diminished cortisol responses, some other studies (Breier et al 1992; Breier et al 1993; Breier et al 1988b; Elman et al 1998) using the metabolic stress paradigm did not find significant differences between patients with a diagnosis of schizophrenia and controls with regard to cortisol changes. Inconsistencies in the results may relate to greater interindividual differences in cortisol levels, as well as to methodological issues (study design, sample size, statistical procedures). The important conclusion that can nevertheless be drawn, is that the DA/NA mediated response to stress is dissociated from the HPA/cortisol response, similar to the findings reported by Breier (Breier et al 1993)

In conclusion, increased HVA response and possibly blunted cortisol response may both reflect dysfunctional adaptation to stress at the biological level. These dysfunctional stress responses may render individuals susceptible to developing psychotic symptoms under stressful conditions. As the results suggest that the alterations in these neurobiological systems under stress are genetically mediated, the findings are compatible with a model of genotype-environment interaction in schizophrenia (van Os et al 1998).

If there is a genetically mediated stress-sensitivity which is centrally regulated and thus associated with higher brain function, the question is where it can be located in the brain. Breier and colleagues (Breier et al 1993) found that the 2DG induced HVA elevation, but not cortisol, was associated with a lower prefrontal cortex volume, suggesting that deficits in the prefrontal cortex may lead to abnormal DA activity. This would be compatible with evidence suggesting that prefrontal dopaminergic neurons inhibit subcortical dopamine activity, and the hypothesis that schizophrenia is characterized by abnormally low prefrontal dopamine activity (causing deficit symptoms) leading to excessive dopamine activity in mesolimbic dopamine neurons (causing positive symptoms) (Davis et al 1991). Future research on correlations between these functional abnormalities in stress response and structural brain changes in both patients with a diagnosis of schizophrenia and their first-degree relatives may help to further elucidate the biological underpinnings of schizophrenia vulnerability and its genetic contribution. More research is also needed to answer the question whether this altered neurobiological response to *metabolic* stress can

be generalized to other external stressors, such as psychological stress, and the clinical relevance of dysfunctional adaptation in daily life.

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Evidence for a cerebellar structural endophenotype in psychosis

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ABSTRACT

Objective. Structural cerebral abnormalities are frequently observed in schizophrenia. These abnormalities may indicate vulnerability for the disorder, as evidenced by reports of familial clustering of measures identified through region-of-interest analyses using manual outlining procedures. We used a novel technique permitting the detection of structural differences within the entire brain to further examine possible structural endophenotypes.

Methods. Magnetic resonance imaging scans were obtained in 31 psychotic patients, 32 non-psychotic first-degree relatives of psychotic patients and 27 healthy controls. The images were processed using an automated procedure, yielding global grey matter, white matter, CSF and total brain volume. The relative distribution of grey matter was compared between groups on a clustered-voxel-wise basis.

Results. Global grey matter and total brain volume did not differ between the groups. White matter volume was significantly higher and CSF volume significantly lower in relatives compared to both cases and controls. The clustered-voxel-based group comparison yielded evidence for significant grey matter deficits in fronto-thalamic-cerebellar regions, in psychotic patients, whereas the most prominent deficits in relatives involved the cerebellum.

Conclusions. Patients with psychosis and first-degree healthy relatives of patients with psychosis show cerebellar abnormalities, which may constitute a marker of genetic transmission.

INTRODUCTION

Many questions remain regarding the origins of the structural cerebral abnormalities associated with schizophrenia. The structural alterations may be directly related to the clinical phenotype and/or be indicators of (genetic) liability for the disorder (endophenotypic markers of liability). Studies of first-degree relatives are useful in the search for such endophenotypes. In addition to evidence for familial aggregation of ventricular enlargement typically derived from computed tomography scanning (reviewed in (Cannon et al 1994a), recent magnetic resonance imaging (MRI) studies show more diverse patterns of cortical and/or subcortical alterations in both patients with schizophrenia and their first degree relatives (Cannon et al 1998; Seidman et al 1997; Seidman et al 1999; Sharma et al 1997; Staal et al 1998). Traditional MRI studies have been based on *a priori* defined regions of interest (ROI) and manual outlining procedures to assess volumetric measurements. This method, however, may preclude the observation of significant but unexpected findings and may have contributed to inconsistencies in the literature and publication bias (Wolkin et al 1998). The availability of various novel techniques that permit the detection of structural differences within the entire brain (Andreasen et al 1994a; Bullmore et al 1999; Collins et al 1994; Wolkin et al 1998; Wright et al 1995) may possibly lead to more consistent results regarding the origins of cerebral abnormalities in schizophrenia and their possible role in the pathophysiology. In addition, these techniques are generally more automated and faster than the traditional ROI methods of analysis, providing the opportunity to investigate larger samples. In the present family study, such a novel technique was used, which comprised both global and regional (clustered-voxel-based) comparisons of the relative distributions of the separate brain tissues. The present paper will focus on grey matter, for which we hypothesised that both cortical and subcortical abnormalities would be present in psychotic patients, and (to a lesser extent) in non-psychotic first-degree relatives of patients with psychosis.

METHODS

Study sample

MRI scans were acquired from 31 patients with psychosis, 32 non-psychotic first-degree relatives of patients with psychosis and 27 healthy controls. The present subsample is part of a larger study, The Maastricht Psychosis Study (Krabbendam et al 2001).

Patients with a life-time history of clinical psychosis (of at least two weeks) according to the RDC criteria (Spitzer et al 1978), who were not in need of in-patient treatment, were recruited at the community mental health centre in Maastricht, the Netherlands.

Non-psychotic first-degree relatives were recruited through the participating patients, as well as through a local relative association. Relatives had to be free from a lifetime history of psychosis. The present sample included 11 families, of which 10 contributed 1 patient

and 1 discordant sibling, and 1 family contributed 2 non-psychotic relatives (parent and sib). In addition, 20 independent first-degree relatives, as well as 21 independent patients were included.

Unrelated healthy controls were sampled from the general population, using a mailing procedure to randomly selected households in the local catchment area. Controls were excluded whenever they had a personal or family history of psychosis or other psychiatric disorder requiring hospital admission.

Other inclusion criteria for all participants (cases, relatives and controls) were: being in the age range of 18-55 years, and being in good health as determined by a physical examination, electrocardiography and routine laboratory investigations. Individuals with a history of severe head trauma, neurological disorders and/or other medical disorders that might have significantly affected brain function or structure were excluded, as well as individuals who used alcohol in excess of five standard units per day or illicit drugs on a weekly basis.

Clinical and diagnostic procedures

Patients, relatives and controls were interviewed with the BPRS (Lukoff et al 1986; Overall et al 1962), the PANNS (Kay et al 1987) and additionally screened for symptoms listed in the OCCPI (McGuffin et al 1991). Where necessary, additional information was derived from case notes and interviews with the responsible medical officer. Using the combined information, the computerised program OPCRIT (McGuffin et al 1991) yielded the following RDC-diagnoses, in the cases: schizophrenia ($n=25$) and schizo-affective disorder ($n=6$) and in four first-degree relatives a (life-time) diagnosis of major depression. No other psychiatric disorders were detected in the relatives, nor were any of the controls diagnosed with psychiatric illness. Handedness was assessed using the Annett Handedness Scale (Annett 1970). To determine life-time history of alcohol and drug use, the appropriate sections of the Composite International Diagnostic Interview (CIDI) (Smeets et al 1993) were used.

Demographic characteristics did not differ between the three groups in terms of age, sex, handedness, height and parental level of occupation (see Table 1). Significant differences between the three groups were found for the number of years in education ($F=3.31$, $df=2$, 87 , $p=.04$) and for educational achievement ($F=4.65$, $df=2$, 87 , $p=.01$). Mean number of years of formal education was higher in the group of relatives compared to both cases and controls who were balanced in this respect. Mean level of educational achievement was lowest in the patient group with no difference between relatives and controls.

The mean duration of illness in the patients was 8.5 years ($SD=5.8$). Twenty-eight patients were receiving antipsychotic medication. The groups did not differ on mean alcohol intake

(in units per week) over the last year (patients: 9.6 (SD=12.5), relatives: 5.0 (SD=8.0), controls: 5.5 (SD=5.8), $F=2.29$, $df=2, 87$, $p=.11$).

All the subjects gave written informed consent after all procedures had been fully explained conform the local ethic's committee guidelines.

Table 1. *Demographic characteristics of the study sample.*

	Cases (s.d.) N=31	Relatives (s.d.) n=32	Controls (s.d.) N=27	F or χ^2 Statistic	P
Gender Male	15	14	12	0.2	.93
Female	16	18	15		
Age, yrs	30.7 (7.5)	35.5 (10.0)	35.5 (9.8)	2.8	.07
Handedness (total of 14-item questionnaire score)	23.7 (6.0)	23.1 (8.2)	23.5 (6.7)	0.1	.93
No. of educational years	13.4 (2.9)	15.0 (3.0)	13.3 (2.6)	3.31	.04
Level of educational achievement.	3.7 (1.3)	4.8 (1.7)	4.3 (1.5)	4.65	.01
Paternal LOA	4.1 (1.7)	3.8 (1.1)	3.4 (1.5)	1.64	.20

Image acquisition

MRI scans were obtained at the Department of Radiology, University Hospital Maastricht, The Netherlands, with a Gyroscan NT T-11 (Philips Medical Systems) operating at 1.5 Tesla. Three millimetre thick interleaved two-dimensional dual-echo fast spin-echo images (60 slices, 0.3 mm gap between slices) were acquired and angled parallel to the clivus, covering the entire brain. Proton density-weighted and T2-weighted images were acquired simultaneously (echo time (TE)₁ = 20 ms, TE₂ = 100 ms, TR = 4000 ms, echo train length: 6, total acquisition time: 10 mins 12 s). The matrix size and field of view was set at 256 x 256 and 22cm respectively. The number of signal averages was 1.

Image processing

Image processing and computations were done on a SUN Ultra10 (Sun Microsystems Inc., Mountain View, CA, USA) workstation. Initially, extracerebral tissues were removed with an automated computational algorithm which uses linear scale-space features derived from the proton density weighted images (Suckling et al 1999a). Each voxel in the intracerebral tissue was then categorised in terms of the proportion of each voxel occupied by grey matter, white matter, CSF or dura/blood vessels using an automated software system. This partitioned the feature space formed by the two MR echoes (proton density and T2-weighting) using a four-class modified fuzzy clustering scheme and assigned continuous membership of each class to every voxel (Suckling et al 1999b). The resultant grey matter

probability maps were then mapped onto a template image in standard stereotactic space (Talairach et al 1988), using affine transforms. The template image was created from the mean of 5 proton density-weighted images from healthy volunteers mapped into standard space using a landmark-based procedure (AFNI, Wisconsin Medical School, WA).

To deal with local misclassification of grey matter resulting from non-uniformity of image contrast along the axis of the magnet bore as the edges of the transmitting/receiving coil are approached, a moving window regime was implemented in the segmentation procedure. For a detailed description see Suckling et al (Suckling et al 1999b).

Statistical analysis

Group differences in the three main brain tissue volumes (grey, white and CSF) and total corrected brain volume ($TCBV = \text{grey} + \text{white} + \text{CSF}$) were analysed using multiple regression. Age, sex, handedness and total corrected brain volume were used as covariates for grey, white and CSF volumes, whereas age, sex and handedness were used as covariates for total corrected brain volume.

After mapping of the grey matter probability maps onto a template image in standard space, the *relative* distribution of grey matter was compared between groups at the single voxel level with a generalised linear model which included the potential confounders global tissue volume, age, sex, and handedness as covariates. This generated maps of the coefficient of group membership divided by its standard error, and the probabilistic significance of this value at each voxel was determined by a two-tailed test using a reference null-distribution formed by a randomisation procedure (Bullmore et al 1999). As structural differences may be subtle and diffuse or focal, as well as testing for between group differences at the single voxel level, tests were also performed at the level of voxel clusters. Maps of the standardised group membership coefficient for the observed and randomised distributions were thresholded such that only voxels with values $> \pm 1.96$ (two standard-deviations from the mean) were retained. The sum of the suprathreshold voxel statistics for each of the resulting three-dimensional clusters was then parameter of interest, sign indicating a relative excess or deficit in tissue density. Significance testing of the clusters was performed against a null-distribution obtained again by randomisation. Validation of nominal Type I error control for the cluster randomisation test has been described in detail by Bullmore and colleagues (Bullmore et al 1999).

In the transformation into standard space, a (partial) correction for global tissue volume takes place, but for comparative purposes, separate analyses were performed treating the whole brain sum of grey matter as a confound in the model.

RESULTS

Global volume differences.

There was no significant main effect for group on either total intracranial volume (patients vs. controls: $\beta = -8.74$, $t = -0.30$, $df = 1$, $p = .77$; relatives vs. controls: $\beta = 35.2$, $t = -1.25$, $df = 1$, $p = .22$) or grey matter volume, although the patients and relatives tended to have lower grey matter volumes than the control group (patients: $\beta = -7.78$, $t = -1.14$, $df = 1$, $p = .26$; relatives: $\beta = -6.48$, $t = -0.97$, $df = 1$, $p = .33$), after adjustment for age, sex and total corrected brain volume. In the relatives, white matter volume was significantly increased compared to both the control group ($\beta = 21.4$, $t = 2.70$, $df = 1$, $p = .008$) and the patient group ($\beta = 22.2$, $t = 2.81$, $df = 1$, $p = .006$), and total (intraventricular and extracerebral) CSF volume was significantly decreased (relatives vs. controls: $\beta = -15.0$, $t = -2.29$, $df = 1$, $p = .02$; relatives vs. patients: $\beta = -23.4$, $t = -3.62$, $df = 1$, $p = .001$). (Table 2.)

Table 2. Mean volumes of grey matter, white matter, CSF and total brain volume after automated segmentation procedures.

	Cases	Relatives	Controls
Grey matter	559.5 (64.0)	572.3 (56.4)	564 (45.1)
White matter**	545.4 (63.4)	588.3 (71.2)	552.7 (53.4)
CSF*	169.2 (32.0)	157.5 (30.4)	166.3 (38.3)
TCBV	1274.1 (127.2)	1317.9 (133.4)	1283.7 (116.9)

** relatives vs. controls: $\beta = 21.4$, $t = 2.70$, $df = 1$, $p = .008$; relatives vs. patients: $\beta = 22.2$, $t = 2.81$, $df = 1$, $p = .006$

* relatives vs. controls: $\beta = -15.0$, $t = -2.29$, $df = 1$, $p = .02$; relatives vs. patients: $\beta = -23.4$, $t = -3.62$, $df = 1$, $p = .001$

Results from the regression analyses for grey matter, white matter and CSF volume were adjusted for age, sex and total corrected brain volume (TCBV = grey + white + CSF - dura, arteries and vessels). Results concerning TCBV were adjusted for age and sex.

Cluster based comparison

The results of using cluster mass to test (by randomisation) for differences between psychotic patients and controls are shown in Fig. 1. Differences between non-psychotic relatives and controls are shown in Fig. 2, and differences between patients and relatives are shown in Fig. 3. The clustered voxels are superimposed on the PD-weighted grey matter template image. Clusters indicating deficits in tissue density are coloured yellow, whereas clusters indicating excesses are coloured purple. Clusters were conservatively thresholded with $p \leq 0.005$.

Adjustment for global tissue volume did not substantially affect the results. The results presented here are all adjusted for global tissue volume. In Table 3, the location of each cluster's centroid is given in Talairach coordinates as well as the size of the clusters in number of voxels.

1. Case-Control comparison

In the case-control comparison, the number of observed suprathreshold clusters by thresholding with $p \leq 0.005$ was: excess: 1, deficit: 6. Thus, a significant difference between controls and patients in grey matter density was identified at 7 spatially extensive three-dimensional voxel clusters. Six clusters indicated *reduced* grey matter density in the patients: i) a cluster including the caudate nucleus and the amygdala (left), ii) a cluster including the cingulate gyrus, central gyrus and medial frontal gyrus (left), iii) a cluster extending from the inferior frontal gyrus (opercular part) to insula (right), iv) a cluster in the right thalamus (centroid in the dorsal medial nucleus), and v) two clusters in the cerebellum (hemispheres, bilateral). *Increased* grey matter density in patients vs. controls was found in one cluster including putamen and globus pallidum (right). (Fig. 1)

2. Relative-Control comparison.

In the relative-control comparison, the results of thresholding cluster mass with $p \leq 0.005$ was 6 (excess: 1; deficits: 5). One cluster indicating grey matter *deficit* was found in the temporal lobe (fusiform gyrus) (right) and the other four deficit clusters were found bilateral in the cerebellar hemispheres. There was one cluster indicating a grey matter *excess* which was found in the superior frontal gyrus (left). (Fig. 2)

3. Case-Relative comparison.

When the group of patients were compared with the group of relatives using paired statistical tests (threshold $p \leq 0.005$), 5 suprathreshold clusters were observed (4 deficits; 1 excess). Thus,

a significant difference between relatives and patients in grey matter density was identified at 5 spatially extensive three-dimensional voxel clusters. The clusters representing *deficits* included: i) the frontal limbic area and superior frontal gyrus (left), ii) insula and inferior frontal gyrus, iii) cingulate gyrus (left), iv) and paracentral lobule (left). One cluster indicating *increased* grey matter density was found which included putamen and globus pallidum. (Fig 3.)

Significant results using cluster mass to test (by randomization) for grey matter differences between 31 psychotic patients and 27 control subjects (*Fig. 1*), between 32 non-psychotic first-degree relatives of psychotic patients and 27 control subjects (*Fig. 2*), and between 31 psychotic patients and 32 non-psychotic first-degree relatives (*Fig. 3*). The observed clusters characterize relative grey matter density differences between the two groups. Clusters indicating deficits in tissue density are colored yellow, whereas clusters indicating excess areas are colored purple. The cluster-wise probability of Type I error was set at $p=0.005$. Results are adjusted for total tissue volume, age, sex, and handedness.

Table 3. Regional grey matter density differences. The location of each cluster's centroid is given in Talairach coordinates (x , y and z (mm)), N = number of voxels in each cluster. The cluster-wise probability threshold was $p = 0.005$

Case-Control comparison

Cerebral region	Side	N	X	Y	Z
Grey matter deficit					
Caudate nucleus, amygdala	L	1051	4.8	18.6	6.6
Cingulate gyrus, central gyrus, medial frontal gyrus	L	576	0.5	17.2	42.2
Inferior frontal gyrus (opercular part), insula	R	745	-35.7	9.9	12.9
Thalamus: dorsal medial nucleus	R	208	-7.3	-16.1	7.2
Cerebellum	R	1605	-26.0	-53.6	-55.9
Cerebellum	L	1253	22.0	-55.9	-59.1
Grey matter excess					
Putamen, globus pallidum	R	1649	-25.4	-1.8	14.8

Table 3. continued*Relative-control comparison*

Cerebral region	Side	N	X	Y	Z
Grey matter deficit					
<i>Temporal lobe (fusiform gyrus)</i>	R	269	-36.7	1.6	-22.6
<i>Cerebellum</i>	R	2034	-25.0	-42.2	-62.2
<i>Cerebellum</i>	R	893	-19.4	-55.9	-48.8
<i>Cerebellum</i>	L	1519	20.9	-56.1	-55.3
<i>Cerebellum</i>	R	609	-2.2	-78.6	-32.3
Grey matter excess					
<i>Superior frontal gyrus</i>	L	447	1.3	-14.8	54.7

Case-relative comparison

Cerebral region	Side	N	X	Y	Z
Grey matter deficit					
<i>Frontal limbic area, superior frontal gyrus</i>	L	2256	1.6	3.1	54.3
<i>Insula, inferior frontal gyrus (opercular part)</i>	R	784	-38.8	3.9	13.0
<i>Cingulate gyrus</i>	L	471	3.2	-41.1	45.5
<i>Paracentral lobule</i>	L	533	4.0	-59.5	21.9
Grey matter excess					
<i>Putamen, globus pallidum</i>	R	1966	-25.5	-4.6	13.6

DISCUSSION

Using a novel comprehensive automated technique applied to structural MRI scans to investigate whole brain global tissue volume and regional group differences in tissue density on a cluster mass basis, the current family study provided evidence for detectable cortical and subcortical grey matter deficits being present not only in psychotic patients but also in non-psychotic first-degree relatives of psychotic patients. Results showed substantial cerebellar grey matter deficits in both groups. In addition, there was evidence for grey matter deficits in the frontal lobe, temporal lobe and thalamus in patients, and to a lesser extent in relatives.

Methodological considerations

The relative-control analysis exhibited less tissue density alterations in fronto-thalamic regions at $p \leq .005$ than the case-control analysis. Nevertheless, there was still a suggestion

for thalamic abnormalities in the relatives at this p-level, because the case-relative comparison did not show any differences in this structure while thalamic deficits in the case-control study were prominent. Moreover, when in post-hoc analyses the cluster-wise probability of Type I error was set at lower thresholds ($p=0.05$), relative versus control comparisons did show significant frontal lobe and thalamic grey matter deficits in addition to cerebellar and temporal lobe deficits (results not presented). Replication studies may need to include more relatives in order to increase power for detecting differences in this group.

Scans were segmented according to the moving window implementation (Suckling et al 1999a) which reduces local misclassification by increasing grey matter in areas where it is systematically underrepresented due to non-uniformity of image contrast. Nevertheless, it cannot be completely ruled out that none of this error remained. However, if existing, such an error is likely to be non-systematic, all the more since the subjects of the three groups were scanned in random order throughout the study period. This would make it difficult to explain why grey matter alterations were found in patients and relatives, but not in control subjects.

Structural abnormalities may be non-specific for schizophrenia and can, for example, be found in mood disorders (Elkis et al 1995). Four relatives had a life-time diagnosis of major depressive disorder according to the RDC. Although it is unlikely that the very similar findings in psychotic patients and non-psychotic relatives would be fully explained by this small subgroup of relatives having (had) a depressive illness, we tested this by excluding them in the cluster analyses. This did not affect the pattern of results.

Excessive alcohol consumption has been found to be associated with cerebellar tissue deficits (Sullivan et al 2000). In the present study, a history of alcohol abuse or dependence was part of the exclusion criteria. Moreover, there were no significant differences between the three study groups in the amount of alcohol used per week, which makes differential alcohol consumption patterns an unlikely explanation for the cerebellar deficits that were seen in patients and relatives.

Findings

Relatives had significantly higher white matter volumes and lower CSF volumes than both cases and controls, whereas cases and controls did not significantly differ on these measures. As enlargement of CSF spaces in schizophrenia is one of the most consistent findings in the neuroimaging literature (Johnstone et al 1976; Raz et al 1990; Shelton et al 1988) the only

non-significant tendency of higher CSF volumes in cases compared to controls was perhaps unexpected. A likely explanation for this is chance, neuroimaging studies sampling around a small effect size. The results pertaining to white matter will be the subject of further investigations using a cluster-statistic based approach. Global grey matter reduction in patients has been demonstrated in several studies (Breier et al 1992; Gur et al 1999; Lim et al 1996; Sullivan et al 1998; Sullivan et al 1996; Zipursky et al 1998; Zipursky et al 1992). In addition, Cannon et al (Cannon et al 1998) reported grey matter reduction in the relatives of patients with schizophrenia, particularly in the frontal and temporal lobes. In the present study, there were no significant differences in grey matter volume between patients and relatives on the one hand, and controls on the other. However, the direction of the effect was towards lower volume in the patient and relative groups. This, in combination with the fact that patients had significantly higher CSF volumes than relatives (possibly indicating the presence of ventricular enlargement), is compatible with the suggestion of Cannon et al (Cannon et al 1994b; Cannon et al 1998) that enlarged ventricles may be a more phenotypic-related abnormality (disease marker), whereas cortical grey matter deficits are shared by family members and may indicate genetic liability for the disorder rather than presence of the clinical syndrome *per se*. In other words, enlarged ventricles upon an already existing genetic vulnerability associated with a pattern of cortical deficits may increase the risk for overt schizophrenia.

Although frontal and thalamic alterations were less prominent in relatives, the current findings provided evidence for fronto-thalamic-cerebellar grey matter deficits, in both psychotic patients and in non-psychotic first degree relatives, suggesting a dysfunctional cortico-cerebellar-thalamic-cortical (CCTC) circuit, as suggested by Andreasen and colleagues (Andreasen et al 1999; Andreasen et al 1998). A disruption in this circuitry may lead to 'cognitive dysmetria', or difficulty with coordinating the processing, prioritisation, retrieval and expression of information (Andreasen et al 1998). Moreover, as non-psychotic

first-degree relatives in this study exhibited similar (but not identical) alterations, deficits in this fundamental circuitry may, at least partly, reflect an association with the genetic factors

that predispose for psychosis vulnerability. The finding of structural deficits in the elements of this cognitive circuitry is compatible with recent evidence for generalised cognitive deficits in schizophrenia (Mohamed et al 1999), and with findings in the present sample indicating that both the cases and relatives performed diffusely worse on a broad range of cognitive tasks when compared with the control group (Krabbendam et al 2001). Other studies have also shown cognitive deficits in relatives of patients (Cannon et al 1994c; Conklin et al 2000; Faraone et al 1995; Finkelstein et al 1997; Keefe et al 1994; Laurent et al 1999; Shenton et al 1989).

Although this study is based on a fairly new method, the observed regional deficits or excesses in grey matter have face validity, as most of the detected regions have previously been found to be associated with (vulnerability for) schizophrenia. For instance, structural frontal lobe abnormalities have been found repeatedly in patients with schizophrenia (Andreasen et al 1986; Andreasen et al 1994b; Breier et al 1992; Buchanan et al 1998; Goldstein et al 1999; Gur et al 2000a), and recent evidence suggest similar abnormalities in non-psychotic siblings (Cannon et al 1998). Temporal lobe abnormalities, particularly in the hippocampal/amygdala area, have been reported in numerous studies investigating patients with schizophrenia (Breier et al 1992; Gur et al 2000b; Nelson et al 1998; Velakoulis et al 1999; Woodruff et al 1997) and also in first-degree relatives of psychotic patients (Cannon et al 1998; Seidman et al 1999). However, findings from recent meta-analyses on frontal lobe (Zakzanis et al 1999) and temporal lobe (Zakzanis et al 2000) studies using structural and functional neuroimaging techniques, suggest a rather moderate prevalence as well as moderate effect sizes of frontal and temporal lobe deficits in schizophrenia.

In the majority of MRI studies investigating the thalamus, an alteration in volume or shape of this structure was noted in patients (Andreasen et al 1994a; Andreasen et al 1990; Buchsbaum et al 1996; Flaum et al 1995; Hazlett et al 1999), and in relatives of patients with schizophrenia (Seidman et al 1997; Seidman et al 1999; Staal et al 1998). The cerebellum has been less well investigated, but has received renewed interest due to the recognition of its role, besides motor functions, in cognition (Rapoport et al 2000), the disturbance of which could be implicated in the pathophysiology and aetiology of schizophrenia (Andreasen et al 1998; Wassink et al 1999). Recent evidence for structural and functional abnormalities of the cerebellum in schizophrenia has come from several studies (Jacobsen et al 1997; Levitt et al 1999; Loeber et al 1999; Nopoulos et al 1999) and was reviewed in (Katsetos et al 1997) and (Martin et al 1995). One group investigated cerebellar abnormalities in relatives using structural MRI, and found a tendency towards volume reduction in the group of all relatives, and significant volume reduction in siblings only (Seidman et al 1999). To our knowledge, the present study is the first in demonstrating structural cerebellar grey matter deficits in non-psychotic first-degree relatives using a voxel based whole-brain analysis.

A recent structural imaging study by Volz et al (Volz et al 2000) using an automatic whole-brain analysis demonstrated reduced volumes simultaneously in the frontal lobe, the temporal lobe, the thalamus, the left cerebellar hemisphere and the right cerebellar vermis in patients with schizophrenia compared with controls.

There is conflicting evidence with regard to alterations in the basal ganglia derived from neuropathological studies (Heckers 1997), but several MRI studies point towards increased volumes of the striatum and globus pallidum (Breier et al 1992; Buchanan et al 1993; Jernigan et al 1991; Swayze et al 1992). Also, in the whole-brain analysis study by Volz et al (Volz et al 2000) a volume increase in the right putamen was found in patients with schizophrenia. These increased volumes seem to be positively associated with exposure to typical antipsychotics (Chakos et al 1994; Corson et al 1999; Gur et al 1998), but other explanations may be possible like defective synaptic pruning of subcortical brain structures (Feinberg 1982) or increased synaptic density as a compensatory mechanism for decreased input from other brain regions (Graybiel 1990). A recent structural MRI study of relatives of schizophrenia patients found a (marginally significant) enlargement in the pallidum and a decreased volume of the putamen, but the patient group was not investigated (Seidman et al 1999). The patients in the present study (but not the relatives), displayed grey matter excesses in the globus pallidum and putamen, concurring with the majority of the studies.

In summary, structural cerebral alterations, particularly in the cerebellum, can be identified in non-psychotic first-degree relatives. As these alterations resemble those in the patients, the suggestion is that they are not illness- or medication-related and likely to be present before illness onset, thereby favouring a neurodevelopmental origin (Murray et al 1987; Weinberger 1987). Therefore, the grey matter deficits found in the present study may constitute markers of genetic transmission, and need further replication both from structural and functional neuroimaging studies in order to elucidate the pathophysiological mechanisms underlying psychosis vulnerability. In addition, structural endophenotypes may become useful candidates as quantitative measures in genetic studies.

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CHAPTER 8

Epilogue

This thesis describes studies on the effects of environmental risk factors, genetic liability and the interplay between them in psychotic disorders. The inability of direct specification or identification of genes involved in the causation of schizophrenia has not precluded substantial progress in knowledge about genetic and environmental risk factors derived from the field of genetic epidemiology. In fact, this line of research has thus far yielded important clues about nature *and* nurture in the etiology of schizophrenia. We have built further on the nature-nurture debate in this thesis. First, we have used a research strategy to get closer to the question whether a presumed (proxy) environmental risk factor for schizophrenia, ie. the degree of urbanization of place of birth, is involved in the causation of the illness. Next, as urban birth and urban residence (later in life) are likely to be highly correlated we developed a method to determine whether this 'urban factor' operates early in the life span or later as knowledge on this might contribute to the identification of the actual risk factor for which 'urbanicity' is a proxy variable. In the next chapter, we examined the association between obstetric complications and family history of psychotic and other psychiatric disorders to test whether there is evidence for genotype-environment correlation. Furthermore, the concept of stress-vulnerability, as a measure of genotype-environment interaction, was examined in a family study by applying a metabolic stress paradigm. Lastly, we have dealt with the question whether there are markers in the brain that may be indicative of genetic liability to schizophrenia.

Summary and implications of the findings

High rates of psychosis in urban areas may be the result of environmental factors associated with urbanization

Urban birth was linearly associated with the risk of developing schizophrenia later in life (Chapter 3). The effect was not specifically related to schizophrenia, ie. there were quantitative differences between the diagnostic categories under investigation, compatible with other evidence suggesting that these different disorders are part of a continuum of psychosis (Van Os et al 1997). The findings are also in line with other evidence for an effect of place of birth on risk for schizophrenia (Freeman 1994; Lewis et al 1992; Mortensen et al 1999; Takei et al 1992; Torrey et al 1990). Nevertheless, the evidence to date, including the present study, was not able to rule out the possibility of genetic effects: although investigating urban birth would preclude a mechanism of predisposed people migrating into cities, 'urban drift' of genetically predisposed parents of patients with schizophrenia could not be excluded. The fact, however, that we observed a change over time in the effect size of the urban risk factor (eg. strongest effect in the latest birth cohorts) is strongly suggestive for an environmental risk factor. The population attributable fraction, eg. the number of cases that could be prevented if the presumed environmental risk factor associated with urbanization could be removed, was around 34%. This figure is quite high and comparable with that in the study by Mortensen and colleagues (Mortensen et al 1999), in which they stated: "although a history of schizophrenia in a parent or sibling is associated with the highest relative risk of having the disease, the place and season of birth account for many more cases on a population basis". Although this line of reasoning is slightly flawed because absence of a family history does not exclude presence of genetic liability, it seems nevertheless of great relevance to search for the causal factor(s) associated with urbanization. Before doing so, it is important to further narrow down the time window during which the urban risk factor operates (see Chapter 4) because urban birth and urban residence are likely to be correlated and it cannot be excluded that urban birth in fact reflects an effect of urban residence at the time of illness onset. This is important to acknowledge because causal factors acting around the time of birth/upbringing may be different in origin than those acting later in life.

*Environmental factors associated with urbanization increase the risk for schizophrenia **before** rather than **around** the time of illness onset.*

For reasons described above, we extended our research by devising a method to discriminate between the effects of urbanization of place of birth/upbringing or the effects of urbanization of place of residence at the time of admission to hospital. In the cohort study described in Chapter 4, the exposure status was defined by a combination of place of birth and place of residence at the time of illness onset in the three most densely populated provinces of the Netherlands (The Randstad, exposed) or all other areas (the non-Randstad, non-exposed). It

appeared that the risk for schizophrenia was strongest in the patients who were born in the exposed area, without an additive effect of being exposed later in life. The study strongly argues against a risk-increasing mechanism around the time of illness onset, but was limited by the fact that the exact point of change of exposure status was unknown, and therefore could not answer the question as to whether the effect of urban birth is confined to the pre- or perinatal period, or extends into the rearing period and early adolescence or even later. No other studies have succeeded in identifying the exact period of risk, although work is underway in Denmark to further examine this issue. There is limited availability of the complex data that are needed for this kind of research, ie. the continuous registration of all individual migration patterns in both the patients and the general population. Several 'early' environmental risk factors associated with the degree of urbanization that may play a role are mentioned in Chapter 4. The evidence, however, is too scarce to draw firm conclusions. There is inconsistency with regard to, for example, an interaction between urban birth and season of birth (Mortensen et al 1999; Takei et al 1995; van Os et al 1996) the presence of which would have supported the urban birth and viral infection theory. Eaton and colleagues (Eaton et al 2000) searched for the relationship between urban birth and obstetric complications and concluded that urban birth was a strong risk factor for schizophrenia, however not mediated by obstetric complications, because the strength of the association between urban birth and schizophrenia was unaffected by adjustment for obstetric complications. As the urban effect appears to have its impact during urban upbringing rather than adult residence per se, developmental mechanisms should also be examined. A possible developmental mechanism whereby social factors may create enduring liabilities for adult psychosis are the effects of the wider social environment, such as the neighbourhood environment, on child and adolescent development (Leventhal et al 2000). Mental states are reactive to experience, and differences in the level of deprivation and social isolation of the neighbourhood environment in urban areas have been shown to be associated with variation in a range of mental health outcomes from problem behaviour in children (Kalff et al 2001) to incidence of neurosis and schizophrenia (Driessen et al 1998; van Os et al 2000). High level of deprivation and low levels of social capital (Kawachi et al 1997) in the wider social environment may enhance the development of "at-risk" mental states that in turn may facilitate the onset of clinical psychosis in adult life. As it is unlikely that existing data at this moment, at least in the Netherlands, can shed more light on this topic, only extensive prospective studies could lead to more answers.

Affective illness increases the risk for exposure to obstetric complications.

As described in Chapter 5, positive associations between OCs in psychotic and control probands on the one hand and familial morbid risk of affective disorder on the other, were found. We did not find evidence for the hypothesis that the genes contributing to the schizophrenia genotype also influence the risk for exposure to OCs, which is compatible with

prior literature on this topic (Cannon 1997; McNeil 1991). Nevertheless, the findings still suggest a mechanism that may be compatible with a model of genotype-environment correlation: the affective disorder genotype may influence the risk for environmental exposure leading to the schizophrenia phenotype. As environmental factors cannot be directly influenced by DNA, genetic control of exposure to such risk factors must be mediated by some characteristics of the person (Plomin 1994). As the associations were confined to the mothers of the patients and controls, the genetic control of exposure to OC's could be mediated by the liability to depression in the mothers of the children that later develop schizophrenia. Mechanisms to explain this phenomenon would be lower compliance of depressive mothers with antenatal care, use of medication, smoking and alcohol use habits, poor weight gain, etc. Rayl and colleagues (Rayl et al 1996), indeed found an association between altered compliance with prenatal care and pre-eclampsia or breech presentation. Other mechanisms one might think of are altered hormonal/biochemical response due to the depressive illness (Orr et al 1995). The present findings are supported by evidence from other studies, suggesting a relationship between maternal depressive symptoms and poor pregnancy outcomes (Hedegaard et al 1993; Jones et al 1998; Orr et al 1995; Sacker et al 1995). It is from a clinical perspective of interest to know what proportion of all OCs can be attributed to the factors that contribute to the familial aggregation of depressive symptoms. It appeared that only 5.6% of the excess number of OCs in patients with psychosis can be explained by these factors. The present study found the highest relative risks for pre-eclampsia and breech presentation when investigating individual OCs. As fetal hypoxia has been proposed as the potential aetiological mechanism that may increase the risk of schizophrenia (Geddes et al 1999; Zornberg et al 2000), and pre-eclampsia is known to also induce fetal hypoxia, it can be speculated that pre-eclampsia may mediate some of the relationship between family history of affective disorder and psychosis.

Altered neurobiological stress response is mediated by the genetic risk for schizophrenia.

Patients with schizophrenia have been found to have altered stress response at the biological level, ie. altered HVA response to metabolic stress (Breier et al 1993). The stress-vulnerability model is a well known concept of disease etiology in psychiatric disorders (Zubin et al 1977). It is, therefore, attractive to hypothesize that the sensitivity to stress is genetically transmitted, as implied by this model. In Chapter 6 this issue was investigated and support for the hypothesis was found in that both patients and first-degree relatives exhibited an altered neurobiological response (increased plasma HVA and blunted plasma cortisol levels) to a metabolic stressor as compared to controls. The strength of this study design was that the 'environment' (ie. metabolic stress) was manipulated and therefore held constant for every study participant. Moreover, stress reactivity was not only subjectively assessed, but was also measured by means of objective blood markers. First-degree relatives showed an intermediary stress response to that of patients and controls, which, in a study design with

minimal 'environmental noise', is highly suggestive of genetic influence. Moreover, the intermediate position of the relatives with regard to the stress measures could not be explained by medication effects, as both relatives and controls were non-medicated. There is hardly any evidence on altered stress response in relatives with regard to these neurobiological measures. An increased dopaminergic stress response in patients has been described earlier (Breier 1989; Breier et al 1993), whereas a blunted cortisol response in schizophrenia has also been reported in prior research using metabolic or psychological stress conditions (Albus et al 1982; Breier et al 1988; Jansen et al 1998). In terms of clinical relevance, the present study has limited direct relevance as the effect sizes of the measures that were used were too low to be able to identify persons with vulnerability to psychosis in the general population. The fact that the stress measures could discriminate between the groups under study may be the consequence of the high prevalence of (vulnerability to) schizophrenia in these groups. Nevertheless, the findings may in a *pre-preventive* way contribute to a better treatment of schizophrenia. Future studies may focus on the structural correlates of these functional abnormalities in the stress response. Moreover, it may be worthwhile to investigate whether this type of stress-sensitivity is specifically related to schizophrenia.

Cerebellar grey matter deficits may represent a structural endophenotype

There is still debate on the nature of cerebral alterations found in schizophrenia. In the structural MRI study described in Chapter 7 we found that cerebral grey matter deficits could not only be found in psychotic patients, but also in their non-psychotic first-degree relatives. There is accumulating evidence describing brain alterations in healthy relatives (Cannon et al 1998; Seidman et al 1997; Seidman et al 1999; Sharma et al 1997; Staal et al 1998). However, there is also a lot of inconsistency in the literature. Studies using a voxel-based methodology are only beginning to be published. Perhaps this method may contribute to more consistent results. The most prevailing deficits in the study described in Chapter 7 in both patients and relatives were found in the cerebellum. The cerebellum has long been neglected in schizophrenia research, for the reason that this structure was primarily assumed to be associated with motor functions. A recent review, however, suggests that the cerebellum does play an important role in cognitive processes (Rapoport et al 2000). Moreover, there is evidence to suggest that alterations in the cognitive fronto-thalamic-cerebellar circuit (Andreasen et al 1998), may be part of the underlying pathophysiological mechanism of schizophrenia. In fact, the present study showed evident grey matter density deficits in the frontal lobe, the thalamus and the cerebellum, in patients as compared to controls. In the relatives, this pattern was less obvious. However, even at the most stringent p-values, cerebellar deficits were apparent in both patients and relatives. As the cerebellar deficits resembled those in the patients it is not likely that the findings are due to illness- or medication effects. From this, it can be concluded that cerebellar grey matter deficits may have a neurodevelopmental origin and may represent genetically mediated vulnerability

indicators for the disorder. Of course, before definite conclusions can be drawn, findings need to be replicated in other voxel-based studies. Comparable results in patients (including cerebellar volume deficits) using whole brain analyses were recently published (Volz et al 2000). However, they did not investigate relatives. Apart from replication studies, the investigation of the functional relevance of structural endophenotypes is a hard-needed focus for future research. Not only correlational studies using structural data, but also several types of functional imaging techniques, may help to further elucidate the pathophysiological underpinnings of schizophrenia vulnerability.

Concluding word

From the evidence described in this thesis we may conclude that there are several techniques to isolate specific environmental effects in schizophrenia, as well as gene-environment interaction and gene-environment correlation. However, the precise mechanisms still remain to be elucidated and replication studies are needed. Although family studies and family history studies have been shown to be useful to detect specific genotype-environment relationships, twin studies are a more powerful means to discriminate between environmental and genetic contributions to the phenotype and may continue to add important knowledge to the field in future research, provided sufficient numbers can be recruited, which unfortunately seldom is the case. In addition, once genes that increase the risk for schizophrenia have been identified, it will be possible to directly investigate genotype-environment interaction. A problem that should be recognized is that in most epidemiologic study designs we are not able to model specific gene-environment relationships and we should be aware of the fact that these relationships may have a substantial influence on commonly used epidemiologic measures such as relative risk (RR) measures, as well as on genetic parameters such as heritability (van Os et al 2001). Further work is needed to remedy these insufficiencies and further elucidate the complex nature-nurture interplay in psychotic illness.

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Summary

The term ecogenetics refers to the study of specific gene-environment relationships (Motulsky et al, 1977). Interaction between genes and environment is more than just involvement of both in disease aetiology. There are several biological plausible mechanisms by which genes and environment can co-influence disease outcome. This thesis addresses several research strategies by which the effects of genes, the environment and their interrelationships can be examined.

Chapters 1 and 2 of this thesis give an overview of the field of genetic-epidemiology, with specific emphasis on ecogenetic studies. General principles from the field such as sources of phenotypic variance, genotype-phenotype relationships and heritability are described. The debate on how many genes are involved in schizophrenia is briefly discussed with the conclusion that a model of multiple genes with individual small effects seems to be the most appropriate. In addition, it is pointed out that the usual dichotomous disorders may not have been suitable phenotypes for molecular genetic approaches, and it is suggested that continuously distributed symptom dimensions may be more effective in this respect. Besides the more general issues, specific genotype-environment relationships such as genotype-environment interaction (genetic control of sensitivity to an environmental exposure) and genotype-environment correlation (genetic control of exposure to a high-risk environment) are described, as well as examples of evidence for such relationships. It is argued that increase in knowledge about genes paradoxically will create possibilities for prevention of disease by environmental manipulation in the presence of genotype-environment interaction or correlation. It is concluded that the emerging field of genetic epidemiology appears to have both theoretical, clinical and public health importance with regard to psychiatric disorders. Although progress has been made in the field, the application of ecogenetic research to schizophrenia is still in its early stages. Often used study designs are discussed, as well as possible new strategies for ecogenetic studies.

Chapter 3 is directed at the question whether urban birth is associated with later schizophrenia, whether there is diagnostic specificity and which individuals are most at risk. All live births recorded between 1942-1978 in the Netherlands were followed-up through the National Psychiatric Case Register for first psychiatric admission for psychosis between 1970 and 1992 (n=42115). The results indicated that urban birth was linearly associated with the risk of developing schizophrenia later in life. The effect was strongly, but not specifically so, related to schizophrenia, ie. there were quantitative differences between the diagnostic

categories under investigation. The effect of urban birth was most pronounced in individuals from the most recent birth cohorts, in men and in early onset cases. There was no evidence for an interaction between urban birth and season of birth. The increase of the urban birth effect over time suggests that urban birth is a proxy measure for an *environmental* risk factor. As place of birth and place of later residence are probably highly correlated, it could not be fully excluded that the associations that were found might be confounded by or in essence reflect an effect of *urban residence*.

Chapter 4 elaborates further on the topic of urbanization and schizophrenia. As none of the studies to date have been able to discriminate between an effect of urban birth and urban residence, a new method was devised to address the question whether any effect of urbanization operates *before* or *around* the time of illness onset. Birth cohorts from 1972 until 1978 were followed up through the Dutch National Psychiatric Case Register for first admission for schizophrenia until 1995. The risk for schizophrenia was examined in four different exposure groups based on the combination of place of birth and place of residence in the three most densely populated provinces of the Netherlands (the Randstad, exposed) or all other areas (the non-Randstad, non-exposed). The four groups were: non-exposed born and non-exposed resident, non-exposed born and exposed resident, exposed born and non-exposed resident, and exposed born and exposed resident. The risk for schizophrenia was strongest in the patients who were born in the exposed area, and there was no evidence for an additive effect of being an urban resident. The study strongly argues against a risk-increasing mechanism around the time of illness onset, but is limited by the fact that the exact point of change of exposure status is unknown. Therefore, although closer to the period in which the risk-increasing factor might operate, it remains to be elucidated whether the effect of urban birth is confined to the pre- or perinatal period, or extends into the rearing period and early adolescence or even later.

Chapter 5 reports on a study that investigated whether the genetic liability to schizophrenia also influences the likelihood to experience obstetric complications (OCs), as the nature of the higher frequency of OCs in patients with schizophrenia is still unclear. To examine whether a mechanism of genotype-environment correlation could explain the higher frequency of OCs in schizophrenia, information on OCs in 151 psychotic patients and 100 controls was collected. In addition, family history (FH) of psychiatric disorder in 600 relatives of patients and in 461 relatives of controls was assessed with the FH-RDC and through personal interviews. Cox proportional hazard regression was used to test for associations between OCs and FH. In the patients, familial morbid risk of schizophrenia and related psychoses was not associated with OCs. However, familial morbid risk of affective disorder, and especially affective disorder in the mother, was associated with a history of OCs in patients. In the controls, affective illness in the mother was also associated with specific OCs. The results

suggest that the familial aggregation of affective symptoms in psychotic patients also influence the likelihood to experience OCs. As the risk for OCs was confined to the mothers of probands, it is unlikely that an affective diathesis in the fetus increases the risk for adverse pregnancy and delivery outcomes. More likely is that depressive illness in the mother leads to the heightened risk of experiencing OCs, through either direct (hormonal/biochemical) or indirect mechanisms (behavioural characteristics).

Chapter 6 presents the findings of a study that examined whether a metabolic stress paradigm could be used to elucidate genotype-environment interaction in schizophrenia. For this purpose, a double blind, randomized controlled intervention was conducted. Metabolic stress was induced by intravenous infusion of a glucose-analog 2-deoxy-D-glucose that produces a mild clinical state of hypoglycaemia in 50 patients with psychosis, 51 first-degree relatives without psychosis, and 50 controls. The effects on neurobiological stress systems were assessed by measuring plasma HVA and cortisol during both the metabolic stress condition and a placebo condition and were tested using multilevel random regression techniques. There was a strong increase in both HVA and cortisol during the stress condition. In addition, there was a statistically significant difference in the HVA increase between the groups, in that patients with psychosis appeared to have the strongest increase in plasma HVA, followed by the relatives and finally the controls. On the contrary, controls had the strongest increase in cortisol, followed first by the relatives and then the patients. The results for cortisol were marginally significant. As the findings suggest that the alterations in these neurobiological systems in response to metabolic stress are genetically mediated, they are compatible with a model of genotype-environment interaction in schizophrenia.

Chapter 7 describes a study that deals with the question whether there are brain alterations that may be indicators of the genetic risk for schizophrenia. MRI scans were obtained in 31 patients, 32 relatives and 27 controls. A novel automated segmentation procedure yielded total grey, white matter and CSF volumes. In addition, relative grey matter density differences between the groups were compared using a voxel-based approach to analysis. There were no significant group differences in total brain volume, or global grey matter volume. White matter volume was significantly increased and CSF significantly decreased in relatives as compared to patients and controls. The voxel-based analyses indicated that patients with psychosis had grey matter deficits in fronto-thalamic-cerebellar regions. In addition, first-degree relatives were also revealed to have grey matter deficits in the cerebellum. It was concluded that cerebellar grey matter deficits may represent an endophenotypic marker of schizophrenia. As the brain alterations in non-psychotic relatives resembled those in the patients it is not likely that the findings are due to illness- or medication effects, thereby favouring a neurodevelopmental hypothesis.

In *Chapter 8*, the results and implications of the separate studies of this thesis are reviewed and discussed. There was evidence for i) a specific, but yet unidentified, environmental factor associated with urban birth/upbringing (and not with urban residence at the time of illness onset) that seems to increase the risk of later schizophrenia, ii) a mechanism by which the affective disorder genotype may increase the risk for exposure to OCs leading to the schizophrenia phenotype (genotype-environment correlation), iii) an altered neurobiological response (increased HVA in the presence of blunted cortisol response) to metabolic stress in schizophrenia that may be genetically mediated (genotype-environment interaction), iv) the presence of cerebellar grey matter deficits as a marker for the genetic vulnerability for schizophrenia. It is concluded that the studies in this thesis have shown several ways to investigate genotype-environment relationships, techniques to investigate specific environmental effects as well as indicators of genetic risk. Insufficiencies of specific methodologies and suggestions for future research are mentioned.

Samenvatting

De term ecogenetica verwijst naar de studie van specifieke gen-omgevings relatiepatronen (Motulsky et al, 1977). Interactie tussen genen en omgeving is meer dan dat beiden een rol spelen in de etiologie van ziekten. Er zijn diverse biologisch plausible mechanismen waarbij genen en omgeving kunnen interacteren en ziektestatus kunnen beïnvloeden. Dit proefschrift beschrijft verschillende onderzoeksstrategieën waarmee de effecten van genen, omgevingsfactoren en hun interacties kunnen worden onderzocht.

In *Hoofdstuk 1 en 2* van dit proefschrift wordt een overzicht gegeven van het gebied van de genetische epidemiologie, met specifieke nadruk op ecogenetische studies. Algemene principes zoals bronnen van fenotypische variantie, genotype-fenotype relaties en heritabiliteit worden beschreven. Het debat omtrent de hoeveelheid genen die een rol spelen bij schizofrenie wordt kort bediscussieerd met als conclusie dat een model van multiële genen met kleine individuele effecten het meest adequaat lijkt te zijn. Vervolgens wordt besproken dat het gebruikelijke dichotome ziekteconcept wellicht niet de meest geschikte fenotypes ten behoeve van moleculair genetisch onderzoek oplevert, waarbij gesuggereerd wordt dat continu verdeelde symptoom dimensies effectiever zouden kunnen zijn in dit opzicht. Naast de meer algemene principes worden ook specifieke gen-omgevings relatiepatronen beschreven zoals gen-omgevings interactie (genetische beïnvloeding van de gevoeligheid voor omgevingsinvloeden) en gen-omgevings correlatie (genetische beïnvloeding van de blootstelling aan een omgeving met een hoog risico) en wordt bewijs geleverd voor het bestaan van dergelijke relaties. Er wordt tevens beargumenteerd dat toename in onze kennis omtrent de genen, paradoxaalwijs, mogelijkheden zal creëren voor preventie van ziekten door middel van manipulatie in de omgeving bij het bestaan van gen-omgevings interactie of correlatie. Geconcludeerd wordt dat de ontwikkelingen op het gebied van de genetische epidemiologie met betrekking tot psychiatrische stoornissen niet enkel van belang zijn vanuit theoretisch opzicht, maar ook vanuit klinisch opzicht en ten aanzien van de volksgezondheid. Hoewel voortgang is geboekt, staat de toepassing van ecogenetisch onderzoek op het gebied van schizofrenie nog in de kinderschoenen. Veel gebruikte onderzoeksmethoden, alsmede mogelijkheden voor nieuwe strategieën ten behoeve van ecogenetisch onderzoek, worden besproken.

Hoofdstuk 3 is gewijd aan de vraag of geboorte in de grote stad geassocieerd is met de ontwikkeling van schizofrenie op latere leeftijd. Tevens is gekeken naar de diagnostische specificiteit van dit effect en welke personen het meeste risico lopen. Alle levend geboren tussen 1942-1978 in Nederland werden vervolgd door middel van het Nationaal Psychiatrisch

Casus Register voor een eerste psychiatrische opname voor psychose tussen 1970 en 1992 ($n=42115$). De resultaten lieten zien dat *geboorte* in geurbaniseerd gebied lineair geassocieerd is met het later ontstaan van schizofrenie. Het effect was het meest prominent, maar niet specifiek voor schizofrenie, er waren kwantitatieve verschillen tussen de diagnostische categorieën. Het effect van urbanisatie was het meest uitgesproken voor personen uit de recentere geboortecohorten, voor mannen en voor personen met een vroeg ziektebegin. Er was geen interactie tussen seizoen en geboorteplaats. De toename van het effect van urbanisatie in de tijd suggereert dat de mate van urbanisatie van geboorteplaats een proxy variabele is voor een *omgevingsrisicofactor*. Omdat geboorteplaats en woonplaats sterk met elkaar gecorreleerd zijn, kan niet volledig worden uitgesloten dat de gevonden associaties een reflectie zijn van *wonen* in geurbaniseerd gebied dan wel dat woonplaats heeft opgetreden als confounder.

In *Hoofdstuk 4* wordt het onderwerp urbanisatie en schizofrenie verder uitgewerkt. Omdat geen enkele studie tot dusverre in staat is gebleken om te discrimineren tussen de separate effecten van urbanisatiegraad van geboorte- en woonplaats, werd hiervoor een nieuwe methode ontwikkeld, met als doel het beantwoorden van de vraag wanneer het effect van urbanisatie optreedt: *vóór* of *rondom* het ontstaan de ziekte. Geboortecohorten van 1972-1978 werden vervolgd door middel van het Nederlands Nationaal Psychiatrisch Casus Register voor een eerste opname voor schizofrenie tot en met 1995. Vier exposure groepen werden samengesteld op basis van een combinatie van geboorteplaats en woonplaats in de drie dichtstbevolkte provincies van Nederland (de Randstad, geëxponeerd) of in alle gebieden daarbuiten (niet-Randstad, niet-geëxponeerd). Het risico voor schizofrenie werd onderzocht in deze vier groepen. Gevonden werd dat het risico het grootst was voor personen die waren *geboren* in de Randstad, zonder additief effect van *wonen* in de Randstad (ten tijde van het ziektebegin). Deze studie pleit sterk tegen een risicoverhogend mechanisme rondom het ontstaan van de ziekte, hoewel er een beperking lag in het feit dat het exacte moment van verandering in exposure status onbekend bleef. Ondanks dat deze studie ons weer een stap verder brengt in het identificeren van de periode waarin het risicoverhogende effect optreedt, zal nog moeten worden uitgezocht of het effect van urbanisatie beperkt is tot de pre- of perinatale periode, zich uitstrekt tot een periode van opgroeien in de grote stad, de vroege adolescentie of zelfs later.

Hoofdstuk 5 rapporteert een studie waarin onderzocht wordt of de genetische kwetsbaarheid voor psychose de kans op het ervaren van obstetrische complicaties (OCs) beïnvloedt, daar de oorzaak van het in hogere frequentie voorkomen van obstetrische complicaties bij schizofrenie nog steeds niet duidelijk is. Om te onderzoeken of dit laatste verklaard kan worden door een mechanisme van gen-omgevings correlatie, werd informatie verzameld over het voorkomen van OCs bij 151 patiënten met psychotische en andere psychiatrische

stoornissen en bij 100 controle personen. Daarnaast werd de Family History (FH) voor psychiatrische stoornissen, middels vragenlijsten (FH-RDC) en persoonlijke interviews, bij 600 familieleden van patiënten en bij 461 familieleden van de controlepersonen bepaald. Met de Cox proportional hazard regressie werden associaties tussen FH en OCs getest. In de patiëntengroep bleek familiale belasting voor schizofrenie en andere psychosen niet geassocieerd te zijn met OCs. Daarentegen was de familiale belasting voor affectieve stoornissen (en met name het voorkomen van affectieve stoornis in de moeder), hoger bij patiënten met OCs. In de controle personen werd eveneens gevonden dat affectieve stoornis in de moeder geassocieerd was met specifieke OCs. De resultaten suggeren dat de familiale aggregatie van affectieve symptomen bij psychotische patiënten ook de blootstelling aan OCs beïnvloedt. Omdat de associatie tussen familiale belasting voor affectieve stoornis en OCs zich beperkte tot affectieve stoornis in de moeder, is het onwaarschijnlijk dat een affectieve diathese in de foetus het risico op zwangerschaps- en geboortecomplicaties verhoogt. Waarschijnlijker is dat een depressieve stoornis bij de moeder door middel van directe (hormonale/biochemische) of indirecte mechanismen (gedragstypische) het risico op OCs verhoogt.

Hoofdstuk 6 presenteert bevindingen van een studie waarin het paradigma van metabole stress wordt gebruikt om gen-omgevings interactie bij schizofrenie aan te tonen. Hiertoe werd een dubbel blind, gerandomiseerde interventie gedaan. Bij 50 patiënten met een psychotische stoornis, 51 eerste-graads familieleden van patiënten met een psychotische stoornis en 50 controle personen werd metabole stress geïnduceerd door middel van intraveneuze infusie van een glucose-analoog 2-deoxy-D-glucose dat een milde toestand van hypoglycaemie produceert. De effecten hiervan op neurobiologische stress systemen werden bepaald door plasma HVA en cortisol op 6 tijdstippen te meten tijdens zowel de metabole stress conditie als tijdens een placebo conditie en werden getest met behulp van multilevel random regressie analyses. Er was een sterke stijging waar te nemen tijdens stress in zowel HVA als cortisol. Ook was er een statistisch significant verschil in de HVA stijging tussen de groepen, waarbij patiënten met psychose de sterkste stijging in plasma HVA lieten zien, gevolgd door de groep familieleden en ten slotte de controle groep. De resultaten suggereerden tevens een groepsverschil voor cortisol in de omgekeerde richting (stijging in cortisol bij patiënten < familieleden < controle personen), hoewel statistische significantie op conventioneel alpha nivo uitbleef. De bevindingen suggereren dat de veranderingen in deze neurobiologische systemen in de reactie op stress onder genetische invloed staan en compatibel zijn met een model van gen-omgevings interactie.

Hoofdstuk 7 beschrijft een studie waarin wordt onderzocht of bepaalde structurele hersenveranderingen bij schizofrenie geassocieerd zijn met de genetische vulnerabiliteit voor de ziekte. MRI scans werden verkregen van 31 patiënten met een psychotische stoornis, 32

eerste-graads familieleden van patiënten met psychose en 27 controle personen. Een nieuwe geautomatiseerde segmentatie procedure leverde totale grijze en witte stof volumes en CSF volumes op. Daarnaast werden verschillen tussen de groepen in relatieve grijze stof dichtheid bepaald met behulp van een voxel-based benadering. Er werden geen significante groepsverschillen in het totale brein volume en het totale grijze stof volume gevonden. Witte stof volume was significant hoger en CSF volume significant lager bij familieleden ten opzichte van patiënten. De voxel-based analyses lieten zien dat patiënten met psychose grijze stof tekorten hebben in fronto-thalamo-cerebellaire gebieden. Daarnaast lieten familieleden ook grijze stof tekorten zien in het cerebellum. Er werd geconcludeerd dat grijze stof vermindering in het cerebellum mogelijk een endofenotypische marker is voor schizofrenie. Omdat de hersenveranderingen in niet-psychotische familieleden lijken op die van patiënten is het onwaarschijnlijk dat de bevindingen het gevolg zijn van ziekte of aan medicatie gerelateerde factoren, daarmee een ontwikkelingsneurologische hypothese ondersteunend.

Hoofdstuk 8, de epiloog, vat de resultaten van de verschillende onderzoeken samen en bediscussieert de implicaties. Er werd bewijs gevonden voor: i) een specifieke, hoewel nog niet geïdentificeerde omgevingsrisicofactor voor schizofrenie, die geassocieerd is met geboorte/opgroeien in geurbaniseerd gebied en niet met wonen in geurbaniseerd gebied rondom het ontstaan van de ziekte, ii) een mechanisme waarbij het genotype voor affectieve stoornis het risico op blootstelling aan specifieke OCs verhoogt en kan leiden tot het schizofrenie fenotype (gen-omgevings correlatie), iii) een veranderde neurobiologische respons (verhoogde HVA respons in aanwezigheid van verminderde cortisol respons) op metabole stress die mogelijk genetisch beïnvloed wordt (gen-omgevings interactie), iv) het bestaan van cerebellaire grijze stof tekorten als marker voor de genetische vulnerabiliteit voor schizofrenie. Er wordt geconcludeerd dat de verschillende methodes beschreven in dit proefschrift kunnen worden gebruikt om gen-omgevings relatiepatronen, effecten van specifieke omgevingsfactoren en indicatoren van genetisch risico te onderzoeken. Tekortkomingen van de verschillende methodes worden besproken en suggesties voor toekomstig onderzoek worden gedaan.

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Curriculum vitae

Machteld Marcelis werd op 4 oktober 1969 geboren in Paramaribo, Suriname. Na twee jaar verhuisde zij naar Nederland. Nadat zij in 1988 het VWO diploma aan het St. Ludgercollege te Doetinchem behaalde, ging zij Geneeskunde studeren aan de Rijksuniversiteit Utrecht. Tijdens de coschappen deed zij haar keuzecoschap in de psychiatrie, op de schizofrenie- en onderzoeksafdeling 'Oostbroek' van het Academisch Ziekenhuis Utrecht. In 1996 behaalde zij haar artsexamen en begon vervolgens als assistent in opleiding haar promotieonderzoek bij de vakgroep Psychiatrie en Neuropsychologie, sectie Sociale Psychiatrie en Psychiatrische Epidemiologie van de Universiteit Maastricht. In 1997 verbleef zij twee maanden op het Institute of Psychiatry te Londen. Tijdens de vier jaren dat zij haar promotieonderzoek uitvoerde, verrichte zij tevens klinische werkzaamheden in het team Stemmingsstoornissen van de Sociaal Psychiatrische Dienst van de Riagg Maastricht. Sinds april 2000 werkt zij in het Psychomedisch Streekcentrum Vijverdal in Maastricht in de functie van psychiater in opleiding.

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