Genetics of Cognitive Endophenotypes in Schizophrenia

A Family-based Study

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A Family-Based Study

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Genetics of Cognitive Endophenotypes in Schizophrenia
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(met een samenvatting in het Nederlands)

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Voor Joost, Teile en Marie

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Chapter 1

Introduction

Abstract

Schizophrenia is a complex and chronic disorder that affects approximately 1% of people worldwide.[1,2] Although the burden for patients, their families, and society is considerable, the options for treatment and prevention of schizophrenia are still limited. In the last few decades research has focused on biological aspects of schizophrenia, with a clear shift towards genetic research since the sequencing of the human genome in 2001.[3,4] These efforts have resulted in accumulating evidence for several genomic regions[5] and candidate genes[6] for schizophrenia. However, replication of most findings has been difficult and the best candidate genes explain only a small proportion of the genetic effects. These disappointing findings are in spite of the spectacular development in techniques recently, e.g., the hypothesis free testing of millions of polymorphisms (variants) in the human genome, referred to as genome-wide association studies (GWAs).

One of the main obstacles in the identification of genetic variants for schizophrenia is its heterogeneous diagnostic entity which is clinically relevant, though less appropriate for etiological and genetic research. Therefore, researches have recently focussed on alternative indicators of liability, or endophenotypes. Endophenotypes, or heritable traits associated with an increased risk for schizophrenia,[7] may aid in genetic research in schizophrenia.

Schizophrenia

Symptoms of schizophrenia vary greatly among patients and can roughly be split into two categories. Positive symptoms are characterized by (auditory) hallucinations and/or (paranoid or bizarre) delusions. Negative symptoms include flat affect and emotion, poverty of speech, lack of motivation, social withdrawal, and/or disorganized speech and thinking. Severe cases can display catatonia; being largely mute or remain motionless in bizarre postures. Most patients suffer from significant social or occupational dysfunction. The diagnosis of schizophrenia can be made if active symptoms are present for at least one month in a period of at least six months of disturbed functioning. Onset of symptoms typically occurs in late adolescence and early adulthood. Treatment for schizophrenia involves therapy and medication. Anti-psychotic medication act on dopaminergic, glutamatergic, and serotonergic regulation. Anti-psychotics are not effective in all patients and can have considerable side-effects.

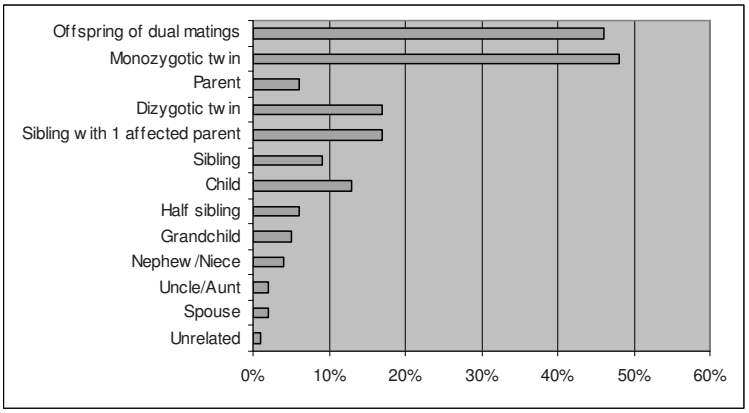
Research in the neurobiology of schizophrenia has converged to the view that schizophrenia is a neurodevelopmental disorder[8] and that neuronal connections are involved, which has been referred to as the dysconnectivity theory.[9,10] These explanations agree with the broad spectrum of impairments displayed in schizophrenia, ranging from deficits in motor, motivational, and affective behaviours to a variety of affected cognitive domains.

Genetics of schizophrenia

Decades of research of family, twin, and adoption studies have revealed a strong genetic component in the liability to schizophrenia.[11-13] Relatives of patients have higher risks to develop schizophrenia with increased risks as more family members are affected and as the degree of kinship increases (Figure 1). For example, children have a risk of 13 % to develop the disorder when one parent has schizophrenia, though 46% when both parents are affected. In monozygotic twins, the concordant diagnosis of schizophrenia (both twins are affected) is more than two times higher (41%–65%) than for dizygotic twins (0%–28%).[14] These findings have yielded estimates of a genetic contribution of up to 80%.[11,12,15]

Although schizophrenia is highly heritable, the illness is not attributable to classical Mendelian dominant or recessive inheritance of a single major gene (for explanations about *inheritance*, *linkage analysis*, and *association analysis*, see below under “Research methods”). Rather, schizophrenia seems multifactorial or polygenetic in origin: multiple genes combine to increase the risk to develop the disorder.[11,16] Most of the genetic variance in schizophrenia is attributable to the sum of multiple weak genetic effects as is evident in meta-analyses of linkage studies[5] and

Figure 1 Lifetime risk to develop schizophrenia (in percentage)[11]



recent genome-wide association studies.[17] Still, highly penetrant mutations can have large effects in occasional families.[18]

Environmental risk factors for schizophrenia are also considerable, such as effects of urban birth, cannabis use, and migrant status.[19] Because gene–environment interactions are included in heritability estimates there may be considerable yet to explore gene-environment effects on the development of schizophrenia. Schizophrenia is thus a complex disorder determined by multiple genes, environment, and their interaction.

So far, meta-analyses have pinpointed a handful of genetic loci for schizophrenia.[5,20,21] Badner and Gershon[21] performed a meta-analysis on 18 genome-wide scan datasets (681 pedigrees) revealing the strongest evidence for susceptibility loci to exist on chromosomes 8p, 13q, and 22q.

Table 1 Ranked linkage loci for schizophrenia from most recent meta-analysis[5]

Rank	Bin	cM	Mb
1	5.6	148.9–178.7	141.8–167.7
2	2.5	117.5–146.9	103.3–134.0
3	1.6	143.1–171.7	114.6–162.1
4	2.8	205.7–235.1	206.3–228.3
5	2.6	146.9–176.3	134.0–169.9
6	1.4	85.8–114.5	57.3–84.6
7	5.7	178.7–208.5	167.7–180.4
8	8.2	28.1–56.2	15.7–32.7
9	10.6	145.9–175.0	123.1–135.1
10	3.4	95.9–127.9	71.6–120.2

Note: the first part of the Bin-number indicates the chromosome followed by the number of the bin (~30cM region) within that chromosome, e.g. 5.6 indicates the sixth bin (149-179 cM) on chromosome 5. CM indicates centimorgan on the Rutger's Map.

Lewis et al.[20] and Ng et al.[5] used a rank-based genome scan meta-analysis to compare the results of published and unpublished data of 20 genome-wide scans. The strongest evidence for linkage was on chromosome 5q and 2q, while neither region reached genome-wide significance. The ten highest ranked linkage loci for schizophrenia are given in Table 1.[5] The high number of suggestive linkage loci and lack of replication indicates the heterogeneity of the disorder.

While the number of candidate genes for schizophrenia is high, identification of susceptibility genes has met with difficulties, as few studies have been able to consistently replicate findings (Table 2).[6,17] Several well-known candidate genes for schizophrenia including DISC1, dysbindin (DTNBP1), and neuregulin 1 (NRG1) have been discovered through their position based on previous linkage regions, indicating the important contribution of linkage studies.[22] The genes most strongly associated with schizophrenia are related to neuronal signalling and neurodevelopment.[10,23]

Summarizing, genetic research in schizophrenia has identified several genomic regions and a range of candidate genes. Findings have been difficult to replicate, resulting in only few well characterised candidate genes. Given this limited success, interest developed in the use of alternative methods. One way is to refine the phenotype, e.g., by using quantitative intermediate phenotypes or endophenotypes.

Table 2 Top ten candidate genes for schizophrenia

Cytogenic region	Gene	Name
1q42.2	DISC1	Disrupted in schizophrenia 1
8p21.3	SLC18A1*	Solute carrier family 18 (vesicular monoamine), member 1
5q34	GABRB2	Gamma-aminobutyric acid (GABA) A receptor, beta 2
11q23	DRD2	Dopamine receptor D2
14q32.32	AKT1*	V-akt murine thymoma viral oncogene homolog 1
12p12	GRIN2B	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B
22q11.21	DGCR2*	DiGeorge syndrome critical region gene 2
1q32.2	PLXNA2*	Plexin A2
16q12.2	RPGRIP1L*	RPGRIP1-like
11p15.3-p14	TPH1	Tryptophan hydroxylase 1

Note: As downloaded from the SZgene website (www.schizophreniaforum.org/res/sczgene/) in May 2009. The list is ranked by effect size based on a continuous meta-analysis of association studies. It only includes genes that contain at least one variant showing a nominally significant summary odds ratio (OR) in the analysis of all ethnic groups, or those limited to samples of Caucasian ancestry. This list represents an up-to-date summary of particularly promising schizophrenia candidate genes that warrant follow-up with high priority, although many of these may represent false-positive findings, in particular those based on small (<10) sample sizes (indicated by an asterisk)[6].

Endophenotypes

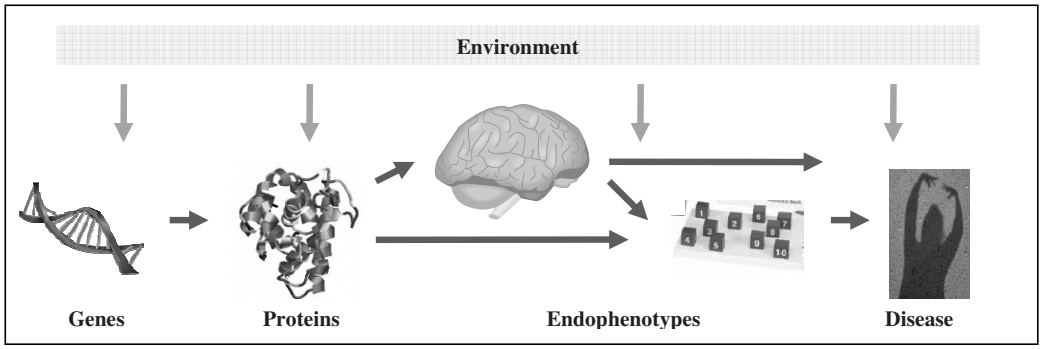
An endophenotype is a physiological or other trait that is related to a disease trait and is measured independently of the disease. Most of the relatives of patients with schizophrenia will be carrying genes associated with the disorder and not be affected by it. Still, these relatives may show deficits on other types of behaviour, e.g., in the cognitive domain, that are related to schizophrenia and its underlying genetic factors. Such heritable neurobiological traits associated with an increased risk for schizophrenia are referred to as endophenotypes.[7] Endophenotypes can be beneficial in genetic research in schizophrenia for having several potential advantages[24]: 1) they may more closely reflect the underlying biological activities of neuronal mechanisms than the disease itself and therefore may more likely reflect major gene effects (Figure 2); rendering these genes more readily detectable; 2) endophenotypes can be measured quantitatively in both patients and their relatives, which may be exploited in more powerful quantitative analysis; 3),to the extent that the neurobiology of the endophenotypes is understood or can be investigated, candidate genes can be identified more readily in linkage areas; 4) endophenotypes lend themselves more directly to animal models.

Several criteria have been formulated for candidate endophenotypes:[7,13] 1) deficits in endophenotypes are associated to schizophrenia; 2) the deficits in endophenotypes are heritable (Ideally, endophenotypes are monogenic, with a clear [Mendelian] mode of inheritance); 3) the endophenotype deficits are reliable and accurately identifiable, i.e., have a good internal consistency, and stable, i.e. being trait-related rather than state-related; 4) the endophenotype and disorder cosegregate; and 5) the endophenotype deficit is present at higher rates in the unaffected biological relatives, and in individuals known to be at high risk for developing the disorder than in the general population.

Endophenotypes for schizophrenia

Schizophrenia can be characterized by deficits in a broad spectrum of behaviours.[25] Consequently, a wide range of endophenotypes have been postulated for schizophrenia. Most candidate endophenotypes are related to the idea that impairments in schizophrenia involve attending, sustaining mental effort over time, retrieving information, and selecting and processing of perceptual information. In order to select potentially useful endophenotypes for this study, a range of cognitive tests were examined for fulfilling the mentioned criteria for endophenotypes. Below, a short description is given for each of the endophenotypes included in this study, providing a concise background explaining the rationale for the selection of tests.

Figure 2 Endophenotype approach



Endophenotypes are intermediate phenotypes that are influenced by the same gene(s) as the disorder, though may reflect more closely the underlying genetic effect. The network of correlations between different endophenotypes may still be complex and multiple genetic (and environmental) effects may contribute.

Inhibition/Gating

Schizophrenia patients have for long been described by others and by themselves as being unable to discriminate between new and old stimuli,[26,27] which seems to be a core feature of the disorder. This difficulty with filtering information can be measured in sensorimotor gating tasks, such as prepulse inhibition and P50 suppression.

Prepulse Inhibition Prepulse inhibition (PPI) of the startle response is a measure of sensorimotor gating and refers to the attenuation of the magnitude of the startle response by a weak sensory (prepulse) stimulus. It enables healthy individuals to focus attention on salient aspects of the environment while screening trivial stimuli. Deficits in PPI are strongly associated with schizophrenia. It is thought that this inability in filtering sensory stimuli in patients with schizophrenia may lead to overstimulation of higher brain regions,[28,29] which may ultimately result in the symptoms of schizophrenia.[30] PPI is impaired in relatives and subjects with schizotypal personality disorder compared to control subjects[31], suggestive of a genetically transmitted deficit. The heritability of PPI is estimated to be 50%.[32] PPI has been shown to be reliable,[33] stable in the normal population,[34] and stable in chronic schizophrenic patients in the absence of changes in clinical state.[35]

P50 Suppression P50 suppression refers to a deficit in the inhibition of the P50 (mid latency auditory) evoked response to repeated auditory stimuli. If two consecutive auditory stimuli are presented, the amplitude of the P50 wave response to the second stimulus will diminish in control subjects, whereas in schizophrenia patients the waves are more similar for both stimuli. This lack of suppression implies a deficit in habituation or sensory gating, also termed 'poor suppression'. The deficit has been reported in half of the relatives of patients with schizophrenia[36] and may be inherited as a dominant trait in some schizophrenia families.[37,38] However, a recent meta-

analysis indicates that later studies have met difficulty in replicating the effects of deviant P50 in relatives.[39] P50 suppression is one of the few endophenotypes that was linked to a chromosomal region. This region on 15q13-14 contains the alpha-7 nicotinic receptor gene, which was shown to be involved in the inhibition deficit.[40,41] The P50 is shown to be heritable.[42] It can be measured reliably[43] and is stable and independent of medication effects.[44]

Visual Perception/Attention

Backward Masking Perceptual processing deficits are commonly observed in schizophrenia. A thoroughly investigated perceptual task in schizophrenia is visual masking. The masking effect involves the disrupted identification of an initial stimulus (the target) by processing of the later stimulus (the mask), by means of a very brief interstimulus interval (ISI).[45] The magnocellular pathway that is used for rapid visual information processing for locating targets was shown to be implicated in the visual masking deficit in schizophrenia.[46-49] Schizophrenia patients show stronger masking effects, as they need longer critical interstimulus intervals (CSI) than controls to identify target stimuli.[50] Masking deficits have been reported in schizophrenia patients who are in clinical remission,[51] in first-degree relatives of schizophrenia patients,[52-54] and in psychosis-prone individuals.[55,56] Backward masking is reliable and stable,[57,58] while heritability estimates are not available.

Continuous Performance Test – Identical Pairs The Continuous Performance Test (CPT)[59] is a typical test of visual sustained attention (vigilance) deficits that seem specific for schizophrenia patients compared to depressed patients and adolescents at risk for affective disorders.[60,61] The CPT identical pairs (CPT-IP) version also taps verbal and spatial attentional processing as well as working memory capacity.[60,62] Impairments on the test have been reported in chronic and first-onset schizophrenia patients,[60,63] in non-psychotic relatives of schizophrenia patients,[64,65] in parents,[66] and in individuals with schizotypal personality disorders.[67] The CPT-IP is among the most cognitively challenging version of the CPT measures. Accordingly, to measure healthy relatives the CPT-IP is preferable, as it measures a higher-processing load, requiring effort in information processing, to avoid possible ceiling effects. The heritability estimate of the measure of attention of the CPT-IP is 49% based on healthy families.[68] The measure has been shown to be reliable and stable.[69]

Trail Making Test The Trail Making Test (TMT) (part B)[70] measures set shifting and motor speed and is considered an attentional task with perceptual and motor components.[71] It seems promising as an endophenotype as it has repeatedly shown to be impaired in schizophrenia patients,[25] in parents,[66,72] and in relatives.[73-75] Heritability has been estimated at 50%.[76] Reliability coefficients reported are around 0.80,[71] though may also be lower.[77] Medication effects are ab-

sent,[78,79] suggesting performance to be stable. An advantage of using the Trail Making Test part B is that its complement, Trail Making Test part A, makes all of the same cognitive demands except set alternation. This eliminates the speed component and allows an estimate of frontally mediated functions.[73]

Perceptual-Motor Speed

Purdue Pegboard Test Decreased motor performance had been associated with schizophrenia even before the introduction of anti-psychotics with side-symptoms affecting motor performance. In children at high risk for schizophrenia, gross motor skills have been related to genetic liability.[80] The Purdue pegboard test measures psychomotor dexterity and seems more specific for schizophrenia, revealed by a larger effect size (0.57) than the related Finger tapping test (0.26) in a study comparing performance of parents of patients with schizophrenia versus controls.[66] Heritability estimates for manual dexterity are not available, while both handedness and motor performance have a significant genetic component.[81,82] The pegboard test was shown to be reliable and stable.[83]

Frontal Brain Function

The prefrontal cortex is associated with higher cognitive functions like problem solving, planning, executive functioning, and working memory, all shown to be impaired in patients with schizophrenia repeatedly[84] as well as in relatives.[85]

Spatial Span The Spatial Span subtest of the Wechsler Memory Scale-III (WMS-III)[86] is a test of spatial working memory and attention and has shown linkage and association to chromosome 1q in patients with schizophrenia and their unaffected co-twins.[87] The backward condition of the task (measuring spatial working memory) showed a heritability of 0.36.[88] Test-retest reliability is 0.74[89] Spatial working memory was also reported to be impaired in schizophrenia patients and their relatives as assessed with a related task (the Delayed Response Task; DRT)[90,91] which was shown to be stable.[92] Spatial working memory seems to reflect specifically the expression of genetic liability to schizophrenia and less so for bipolar disorder.[93]

Digit Span The Digit Span Test is a Wechsler Adult Intelligence Scale (WAIS) subtest[94] with a forward and a backward version that respectively measure verbal attention and verbal working memory. Digit span performance was shown to be affected in schizophrenia patients and relatives,[95,96] though not consistently.[25] Heritability estimates for short term memory range from 30% to 60% and for verbal working memory from 43% to 49%.[88,97] Test-retest reliability ranges from 0.72 - 0.80.[88]

Memory/Temporal Lobe Function

California Verbal Learning Test The California Verbal Learning Test (CVLT) is a test of verbal learning and memory. In a review of 204 studies on neurocognitive deficits in schizophrenia, the largest mean effect size of 1.41 was reported for global verbal memory.[25] Furthermore, in a meta-analysis, memory impairment in schizophrenia was shown to be stable, wide ranging, and not substantially affected by potential moderating factors such as severity of psychopathology and duration of illness.[95] Verbal memory is also consistently found to be affected in relatives and shows the highest specificity in a meta-analytic review on neurocognitive performance in relatives of patients with schizophrenia.[85] A trend for elevated relative risk was reported for verbal memory as measured with the CVLT,[98] suggesting the deficits to be familial and possibly heritable. The split-half reliability of the CVLT is 0.77-0.86.[99]

Verbal fluency Verbal fluency as tested by the Verbal Fluency task[100] has been shown to be impaired in schizophrenia patients[25] and in their relatives.[101,73,102] Verbal Fluency reflects speed of processing and can be parsed into semantic (category) versus phonemic (letter) fluency. Both tasks are associated with left frontal lobe activation, but semantic fluency more with temporal and letter fluency more with frontal regions. In several studies, schizophrenia patients show impairments on both tasks, though especially on semantic fluency.[103] Verbal fluency has a genetic variance of 34%.[76] Test-retest reliability estimates are around 0.70.[71] Stability of verbal fluency over a 4-year period was 0.48 for schizophrenia patients and 0.79 for controls.

Facial Recognition Deficits in facial recognition[104] have been associated with schizophrenia[105] and are attributed to frontotemporal dysfunction.[106] Biological relatives of patients have similar deficits, suggesting a genetic susceptibility.[105] Therefore, face recognition deficits were suggested as an endophenotype for schizophrenia reflecting fronto-temporal impairment. No estimates for heritability are available. Reliability was moderate (0.69), and stability only somewhat higher (0.71).[107]

Intelligence quotient Low (premorbid) intelligence (intelligence quotient; IQ) has been associated with (genetic) risk for developing schizophrenia.[108,109] Specifically, the correlation coefficient between IQ and schizophrenia was estimated to be –0.61, and shared genetic variance may account for 92% of the covariance between these phenotypes.[110] Intelligence represents, by definition, the covariation among diverse measures of cognitive ability[111] and correlates with more elementary cognitive tasks.[112,113] Heritability estimates of IQ have generally been higher, i.e., 50% to 80%,[114,115] than other cognitive endophenotypes, although multiple genes are likely to be involved.[116] Reliability and stability of IQ as measured with the Wechsler Adult Intelligence Scale (WAIS-III)[117] have been high.[117] Four subtests of the WAIS-III, i.e., information, block design, arithmetic, and digit symbol substitution, can be used to estimate IQ. This combination

of subtests has been shown to be the most reliable four-subtests version of estimating IQ in patients with schizophrenia,[118] on the basis of being not time-consuming and including one subtest of all four index scores of the WAIS-III.

Personality/ Schizophrenia Spectrum

Neuroticism Neuroticism has been proposed as a risk factor for schizophrenia,[119] as it contributes to the risk of psychotic or psychosis-like symptoms at 3-year follow-up,[120] suggestive of a shared liability. Twin and adoption studies have produced heritability estimates of about 40%.[121] Furthermore, neuroticism as measured with the NEO-FFI shows internal consistencies between 0.80 and 0.90. Test-retest correlations were above 0.80.[122] Neuroticism does not seem to be specific for schizophrenia; rather it acts as a risk-factor for several psychiatric diseases.

Openness Openness to experience is one of the major dimensions of personality, the Big Five[123] and is often characterized as cognitive flexibility or exploration. Persons with psychotic experiences in the general population score high on openness,[124] suggesting an association to the vulnerability to schizophrenia. Interestingly, openness is the only personality trait linked to the functions of the dorsolateral prefrontal cortex,[125] a structure associated with schizophrenia and dopamine transmission.[126] Patients with schizophrenia tend to score lower on openness,[127,128] with negative symptoms correlating negatively with openness, while positive symptoms show a positive correlation.[129] Indeed, descriptions of closeness (as opposed to openness as described by the NEO-FFI), such as "muted emotional responses," fit with the clinical phenotype of schizophrenia patients having negative symptoms, whereas descriptions of openness, such as "curious about both inner and outer world," accords with positive symptoms, such as hallucinations or delusions. Openness has not been tested in relatives of schizophrenia patients; hence, it is unknown whether openness is related to the vulnerability for schizophrenia. However, openness is highly heritable,[114] reliable, and stable,[122] and may be of interest as an endophenotype for schizophrenia.

Schizotypy Schizotypy is thought to be related to a familial or genetic liability to develop schizophrenia.[12] Schizotypal traits are more common in non-psychotic relatives of schizophrenic patients[130] and correspond to the symptoms of the proband.[131] Moreover, similar cognitive deficits are evident in schizotypal individuals.[132,133] The SPQ questionnaire, particularly positive schizotypy, seems to reflect the biological-genetic vulnerability to schizophrenia.[134] Heritability estimates are not available. The SPQ has an internal consistency of 0.91, and a test-retest reliability of 0.82.

EEG

Oscillatory brain activity (electroencephalogram; EEG) in resting state reflects the activity of various circuits of underlying neurons, is correlated to personality and cognitive features,[135] and is well studied in schizophrenia.[136] Generally, individuals with schizophrenia display increased low frequency (delta and theta waves),[137] decreased alpha waves, and increased beta (high) frequency activity.[138] This slowing of the EEG has been linked to an impaired subcortical synchronization system including the mesencephalic reticular formation, nucleus reticularis, and the thalamus.[139] Oscillatory activity has a high heritability,[140-143] good reliability,[144] good stability,[145-147] and is deviant in both patients[138] and relatives.[148-150] Moreover, QEEG may more closely reflect the underlying genetic effects than behaviour task performance, as has been suggested for other brain activity phenotypes.[151,152]

Genetics of Endophenotypes

With a boost in the last decade, the endophenotype approach has made its way into genetic research of schizophrenia. Before, most studies were aimed at identifying deviant behaviour in the unaffected relatives of patients with schizophrenia.[73,101,153] Parallel with the explosive progress in genetic research since the complete human genome mapping, endophenotypes were easily incorporated into association studies, testing candidate genes for their effects on endophenotypic traits.[154,155] On the contrary, the P50 endophenotype is an example of a trait that had been incorporated in a classical positional cloning approach more than a decade ago. When it was shown that the P50 deficit may be inherited as a dominant trait in some schizophrenia families,[37] an initial genome-wide linkage analysis was performed in these nine families.[38] Given a positive LOD at the 15q13-14 region together with the localisation of a gene of interest (the a7-nicotinic receptor gene, CHRNA7) in this area, a denser genome-wide linkage study was performed including a fine-mapping of the region of interest.[40] A significant linkage peak (LOD 5.3) was observed at the locus, followed by the identification of significant genotype-wise disequilibrium for a marker within a 1-Mb region on chromosome 15 containing CHRNA7 and CHRFAM7 (a highly related gene) in parent-child triads from families with schizophrenia.[41] This endopenotype has thus been successfully implemented in genetic research in schizophrenia, although later studies have met difficulty in replicating the effects of deviant P50 in relatives.[39]

Endophenotype linkage studies

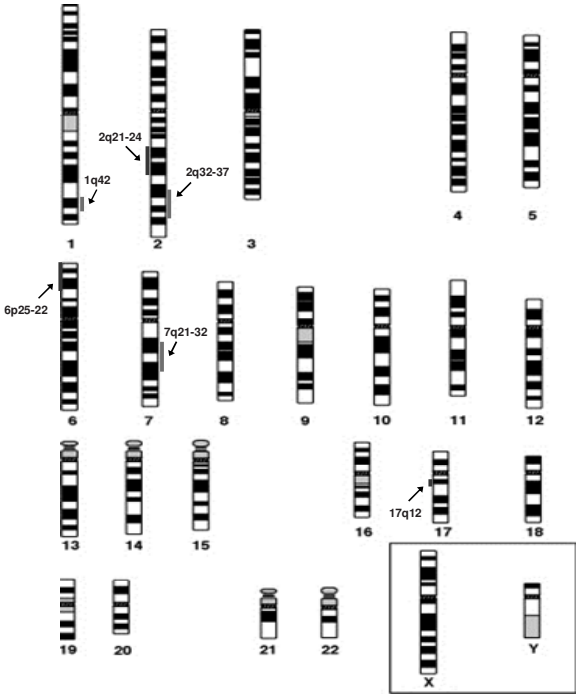
A search for linkage studies using potential endophenotypes for schizophrenia of up to 2009 resulted in the identification of 28 genome-wide linkage studies. This search included a broad

range of putative candidate endophenotypes, including cognitive traits (IQ), cannabis dependence, volumetric changes, and personality. Considering the number of candidate endophenotypes suggested for schizophrenia, the number of linkage studies is relatively limited. Also, the greater part of these studies have focused on electrophysiological traits that were measured in families affected with alcoholism,[156-162] IQ,[159,163,164] or neuroticism.[165-168] When studying the (near) overlap between loci identified for IQ and for schizophrenia, several regions of interest could be identified, i.e., on 1q, 2q, 6p, 7q, and 17q (Figure 3).[20,169-182] Also, some of these regions have been linked to working memory as well.[181,183-185]

Endophenotypes and candidate genes

Functional candidate genes have been tested extensively for endophenotypes, including APOE,[186] COMT,[154,155,187] and BDNF.[188] Although results have not always been straightforward,[155,189] endophenotypes are becoming increasingly integrated in unravelling the genetic-biological pathways that shape schizophrenia.[190,191] Recently, improvements have been made in testing combined genetic effects on cognitive functioning, e.g., testing the combined effects of COMT and GRM3 on dissociable components of the frontoparietal working memory network.[192] Also, genome-wide association studies are appearing for endophenotypes.[193]

Figure 3 Overlap in candidate loci between IQ and schizophrenia (left of chromosome) and additionally working memory (right of chromosome)



Research methods (a mini introduction)

Inheritance

As described above, schizophrenia can be characterized by multifactorial or polygenetic inheritance: multiple genes combine to increase the risk to develop the disorder, i.e., the illness is not attributable to the effect of a single major gene. Such complex inheritance contrasts with classical or Mendelian dominant or recessive inheritance of a single major gene. To explain shortly, the mode of inheritance is dominant if a mutation in only one copy of the gene is sufficient to cause the disorder. If two mutated copies of the gene are necessary for developing a disorder, the mode of inheritance is termed recessive. However, because of, among others, gene-gene interactions, gene-environment interactions, and epigenetic phenomena, most genetic mutations do not produce a Mendelian pattern of inheritance of the phenotype. Hence, most heritable disorders and traits, such as diabetes mellitus, psychiatric disorders, or blood pressure, are characterised by a complex inheritance. Although less well researched, the same is likely to be true for most (quantitative) neurocognitive traits. The proportion of genetic variance can be quantified in twin-adoption-, or family-based studies. In twin models, most of the genetic and environmental components of the total variance of a trait can be estimated, including differentiation of common from unique environment. Common or shared environmental effects cannot be distinguished from genetic effects in family studies. However, by using multigenerational pedigrees, heritability estimates are less likely to be inflated by shared environmental effects than in studies using first-degree relatives only.

Linkage analysis

In human genetic research, roughly two methods are available: linkage analysis and association analysis. Linkage analysis involves locating a region on the genome (locus) where a gene of interest may reside. In search for the locus, multiple markers across the genome are typed in related individuals. If a genetic marker is located close to the disease causing gene, than all affected members in the family are expected to carry the same variant of the marker (in a fully penetrant, Mendelian disorder), while the unaffected individuals do not. Linkage analysis is based on the estimation of the genetic distance (or recombination fraction) between the gene (trait locus) and the genetic marker. During each meiosis event, parental chromosomes recombine when forming gametes, termed crossing over. The more recombination occurs, the smaller the parts coming from the same ancestor that are shared among relatives. The number of recombination events determines the size of the region of linkage, which is typically broad in family data, because of the limited number of meioses and thus recombination events. These broad link-

age intervals can contain dozens to hundreds of candidate genes. Therefore, following linkage analysis, association studies are generally performed using markers within a region or positional candidate gene, to identify the gene of interest in unrelated individuals.

Association analysis

Association analysis is a method for identifying a gene that is associated with the disease in the population. It is based on the same principle of recombination events between the marker and disease-related variant as linkage analysis. If a marker is located very close to a gene, and therefore rarely separated by crossing over, they are said to be in linkage disequilibrium (LD). In unrelated individuals from the general population, the number of recombination events since their common ancestor is substantial, resulting in small parts of the genome with a strong LD, so-called haploblocks. Association therefore requires a high density of markers. Through recent technological developments, genotyping of hundred thousands to a million markers has become feasible and affordable in the large numbers of individuals that are needed to detect the small effect sizes involved in psychiatric disorders. Prior to such hypothesis-free and genome-wide testing, association studies were mainly used for assessing functional candidate genes or positional candidates on the basis of linkage regions. The association test is based on the comparison of allele frequencies among cases and controls. If two groups differ only on a trait of interest, then significant differences in allele frequencies between the groups are likely to reflect a nearby located disease-related variant.

Outline of this thesis

The main objective of this study is to unravel the genetic characteristics of promising endophenotypes for schizophrenia and to apply these in genetic research.
Specifically, we aimed:

1) To identify heritable endophenotypes (**Chapter 2 and 5**);
2) To investigate the genetic characteristics of the heritable endophenotypes (**Chapter 2, 3 and 5**);
3) To identify quantitative trait loci (QTLs) for heritable endophenotype(s) (**Chapter 4 and 5**);
4) To identify potential candidate genes for schizophrenia within the loci (**Chapter 4**);
5) To identify an association between potential candidate genes and schizophrenia (**Chapter 4**).

The first part of this thesis focuses on identifying heritable endophenotypes and describing their heritable characteristics. First, endophenotypes that have been associated with schizophrenia in the literature were selected on the basis of criteria for endophenotypes. **Chapter 2** describes the investigation of heritability and mode of inheritance of 13 endophenotypes in 25 extended families multiply affected with schizophrenia. It was reasoned that useful endophenotypes are heritable and have a clearer (Mendelian) mode of transmission as compared to schizophrenia. Based on their heritable characteristics, the most useful candidate endophenotypes for genetic research were identified. **Chapter 3** described the investigation of genetic sharing among the endophenotypes and IQ, describing to what extend the endophenotypes share genetic and environmental variance.

In the second part, the promising endophenotypes are linked to the genotypes. **Chapter 4** describes the search for genetic loci for heritable endophenotypes in a genome-wide high-density linkage analysis. The endophenotypes were selected on the basis of their heritable characteristics described in the first part. Observed linkage regions were screened for potential candidate genes for schizophrenia by performing prioritization analysis and examining published expression datasets. It was hypothesized that positional candidate genes that are functionally interrelated and related to schizophrenia and are expressed differentially in the brain in schizophrenia are putative candidate genes for schizophrenia that are associated to schizophrenia. Potential candidate genes were tested for association with schizophrenia. **Chapter 5** concentrates on oscillatory activity (EEG) as an endophenotype and follows the approach of the preceding chapters. First, several frequency bands at multiple scalp locations were examined for their heritable characteristics. Subsequently, the heritable traits were included in a genome-wide linkage analysis.

Chapter 6 gives a summary of the chapters followed by a discussion of the findings.

References

1. Bhugra D (2005): The global prevalence of schizophrenia. *PLoS Med* 2: e151.
2. Goldner EM, Hsu L, Waraich P, Somers JM (2002): Prevalence and incidence studies of schizophrenic disorders: a systematic review of the literature. *Can J Psychiatry* 47: 833-843.
3. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG et al (2001): The sequence of the human genome. *Science* 291: 1304-1351.
4. McPherson JD, Marra M, Hillier L, Waterston RH, Chinwalla A, Wallis J et al (2001): A physical map of the human genome. *Nature* 409: 934-941.
5. Ng MY, Levinson DF, Faraone SV, Suarez BK, DeLisi LE, Arinami T et al (2008): Meta-analysis of 32 genome-wide linkage studies of schizophrenia. *Mol Psychiatry* 14: 774-85.
6. Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ et al (2008): Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat Genet* 40: 827-834.
7. Gottesman II, Gould TD (2003): The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 160: 636-645.

8. Fatemi SH, Folsom TD (2009): The neurodevelopmental hypothesis of schizophrenia, revisited. *Schizophr Bull* 35: 528-548.
9. Friston KJ (1999): Schizophrenia and the disconnection hypothesis. *Acta Psychiatr Scand Suppl* 395: 68-79.
10. Harrison PJ, Weinberger DR (2005): Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol Psychiatry* 10: 40-68.
11. Gottesman,II (1991): *Schizophrenia genesis: the origin of madness*. New York: Freeman.
12. Kendler KS, Diehl SR (1993): The genetics of schizophrenia: a current, genetic-epidemiologic perspective. *Schizophr Bull* 19: 261-285.
13. Tsuang M (2000): Schizophrenia: genes and environment. *Biol Psychiatry* 47: 210-220.
14. Cardno AG, Gottesman II (2000): Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. *Am J Med Genet* 97: 12-17.
15. Sullivan PF, Kendler KS, Neale MC (2003): Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* 60: 1187-1192.
16. McGue M, Gottesman II, Rao DC (1985): Resolving genetic models for the transmission of schizophrenia. *Genet Epidemiol* 2: 99-110.
17. Cichon S, Craddock N, Daly M, Faraone SV, Gejman PV, Kelsoe J et al (2009): Genomewide association studies: history, rationale, and prospects for psychiatric disorders. *Am J Psychiatry* 166: 540-556.
18. St Clair D (2009): Copy number variation and schizophrenia. *Schizophr Bull* 35: 9-12.
19. Selten JP, Cantor-Graae E, Kahn RS (2007): Migration and schizophrenia. *Curr Opin Psychiatry* 20: 111-115.
20. Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I et al (2003): Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 73: 34-48.
21. Badner JA, Gershon ES (2002): Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 7: 405-411.
22. Williams HJ, Owen MJ, O'Donovan MC (2009): New findings from genetic association studies of schizophrenia. *J Hum Genet* 54: 9-14.
23. Moises HW, Zoega T, Gottesman II (2002): The glial growth factors deficiency and synaptic destabilization hypothesis of schizophrenia. *BMC Psychiatry* 2: 8.
24. Braff D, Schork NJ, Gottesman II (2007): Endophenotyping schizophrenia. *Am J Psychiatry* 164: 705-707.
25. Heinrichs RW, Zakzanis KK (1998): Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. *Neuropsychology* 12: 426-445.
26. Schmolling P (1983): A systems model of schizophrenic dysfunction. *Behav Sci* 28: 253-267.
27. Shakow D (1963): Psychological deficit in schizophrenia. *Behav Sci* 8: 275-305.
28. Venables PH (1964): Input dysfunction in schizophrenia. In Maher BA, editor. *Progress in Experimental Personality Research*. New York, London: Academic Press, pp 1-47.
29. McGhie A, Chapman J (1961): Disorders of attention and perception in early schizophrenia. *Br J Med Psychol* 34: 103-116.
30. Braff DL, Geyer MA (1990): Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch Gen Psychiatry* 47: 181-188.
31. Cadenhead KS, Swerdlow NR, Shafer KM, Diaz M, Braff DL (2000): Modulation of the startle response and startle laterality in relatives of schizophrenic patients and in subjects with schizotypal personality disorder: evidence of inhibitory deficits. *Am J Psychiatry* 157: 1660-1668.
32. Anokhin AP, Heath AC, Myers E, Ralano A, Wood S (2003): Genetic influences on prepulse inhibition of startle reflex in humans. *Neurosci Lett* 353: 45-48.
33. Abel K, Waikar M, Pedro B, Hemsley D, Geyer M (1998): Repeated testing of prepulse inhibition and habituation of the startle reflex: a study in healthy human controls. *J Psychopharmacol* 12: 330-337.
34. Cadenhead KS, Carasso BS, Swerdlow NR, Geyer MA, Braff DL (1999): Prepulse inhibition and habituation of the startle response are stable neurobiological measures in a normal male population. *Biol Psychiatry* 45: 360-364.
35. Ludewig K, Geyer MA, Etzensberger M, Vollenweider FX (2002): Stability of the acoustic startle reflex, prepulse inhibition, and habituation in schizophrenia. *Schizophr Res* 55: 129-137.

36. Waldo M, Myles-Worsley M, Madison A, Byerley W, Freedman R (1995): Sensory gating deficits in parents of schizophrenics. *Am J Med Genet* 60: 506-511.
37. Siegel C, Waldo M, Mizner G, Adler LE, Freedman R (1984): Deficits in sensory gating in schizophrenic patients and their relatives. Evidence obtained with auditory evoked responses. *Arch Gen Psychiatry* 41: 607-612.
38. Coon H, Byerley W, Holik J, Hoff M, Myles-Worsley M, Lannfelt L et al (1993): Linkage analysis of schizophrenia with five dopamine receptor genes in nine pedigrees. *Am J Hum Genet* 52: 327-334.
39. de Wilde OM, Bour LJ, Dingemans PM, Koelman JH, Linszen DH (2007): A meta-analysis of P50 studies in patients with schizophrenia and relatives: differences in methodology between research groups. *Schizophr Res* 97: 137-151.
40. Freedman R, Coon H, Myles-Worsley M, Orr-Urtreger A, Olincy A, Davis A et al (1997): Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proc Natl Acad Sci U S A* 94: 587-592.
41. Freedman R, Leonard S, Gault JM, Hopkins J, Cloninger CR, Kaufmann CA et al (2001): Linkage disequilibrium for schizophrenia at the chromosome 15q13-14 locus of the alpha7-nicotinic acetylcholine receptor subunit gene (CHRNA7). *Am J Med Genet* 105: 20-22.
42. Young DA, Waldo M, Rutledge JH, III, Freedman R (1996): Heritability of inhibitory gating of the P50 auditory-evoked potential in monozygotic and dizygotic twins. *Neuropsychobiology* 33: 113-117.
43. Cardenas VA, Gill P, Fein G (1997): Human P50 suppression is not affected by variations in wakeful alertness. *Biol Psychiatry* 41: 891-901.
44. Myles-Worsley M (2002): P50 sensory gating in multiplex schizophrenia families from a pacific island isolate. *Am J Psychiatry* 159: 2007-2012.
45. Breitmeyer BG, Ogmen H (2000): Recent models and findings in visual backward masking: a comparison, review, and update. *Percept Psychophys* 62: 1572-1595.
46. Schuck JR, Lee RG (1989): Backward masking, information processing, and schizophrenia. *Schizophr Bull* 15: 491-500.
47. Green MF, Nuechterlein KH, Mintz J (1994): Backward masking in schizophrenia and mania. II. Specifying the visual channels. *Arch Gen Psychiatry* 51: 945-951.
48. Cadenhead KS, Serper Y, Braff DL (1998): Transient versus sustained visual channels in the visual backward masking deficits of schizophrenia patients. *Biol Psychiatry* 43: 132-138.
49. Schechter I, Butler PD, Silipo G, Zemon V, Javitt DC (2003): Magnocellular and parvocellular contributions to backward masking dysfunction in schizophrenia. *Schizophr Res* 64: 91-101.
50. Braff DL (1981): Impaired speed of information processing in nonmedicated schizotypal patients. *Schizophr Bull* 7: 499-508.
51. Green MF, Nuechterlein KH, Breitmeyer B, Mintz J (1999): Backward masking in unmedicated schizophrenic patients in psychotic remission: possible reflection of aberrant cortical oscillation. *Am J Psychiatry* 156: 1367-1373.
52. Green MF, Nuechterlein KH, Breitmeyer B (1997): Backward masking performance in unaffected siblings of schizophrenic patients. Evidence for a vulnerability indicator. *Arch Gen Psychiatry* 54: 465-472.
53. Keri S, Kelemen O, Benedek G, Janka Z (2001): Different trait markers for schizophrenia and bipolar disorder: a neurocognitive approach. *Psychol Med* 31: 915-922.
54. Green MF (2006): Cognitive impairment and functional outcome in schizophrenia and bipolar disorder. *J Clin Psychiatry* 67 Suppl 9: 3-8.
55. Merritt RD, Balogh DW (1989): Backward masking spatial frequency effects among hypothetically schizotypal individuals. *Schizophr Bull* 15: 573-583.
56. Cadenhead K, Kumar C, Braff D (1996): Clinical and experimental characteristics of "hypothetically psychosis prone" college students. *J Psychiatr Res* 30: 331-340.
57. Green MF, Nuechterlein KH, Breitmeyer B (2002): Development of a computerized assessment for visual masking. *Int J Methods Psychiatr Res* 11: 83-89.
58. Rund BR, Landro NI, Orbeck AL (1993): Stability in backward masking performance in schizophrenics, affectively disturbed patients, and normal subjects. *J Nerv Ment Dis* 181: 233-237.
59. Rosvold HE, Mirsky AF, Sarason I, Bransome ED Jr, Beck LH (1956): A continuous performance test of brain damage. *J Consult Psychol* 20: 343-350.

60. Cornblatt BA, Lenzenweger MF, Erlenmeyer-Kimling L (1989): The continuous performance test, identical pairs version: II. Contrasting attentional profiles in schizophrenic and depressed patients. *Psychiatry Res* 29: 65-85.
61. Cornblatt B, Obuchowski M, Roberts S, Pollack S, Erlenmeyer-Kimling L (1999): Cognitive and behavioral precursors of schizophrenia. *Dev Psychopathol* 11: 487-508.
62. Cornblatt BA, Keilp JG (1994): Impaired attention, genetics, and the pathophysiology of schizophrenia. *Schizophr Bull* 20: 31-46.
63. Cornblatt B, Obuchowski M, Schnur DB, O'Brien JD (1997): Attention and clinical symptoms in schizophrenia. *Psychiatr Q* 68: 343-359.
64. Franke P, Maier W, Hardt J, Hain C, Cornblatt BA (1994): Attentional abilities and measures of schizotypy: their variation and covariation in schizophrenic patients, their siblings, and normal control subjects. *Psychiatry Res* 54: 259-272.
65. Laurent A, Saoud M, Bougerol T, d'Amato T, Anchisi AM, Biloa-Tang M et al (1999): Attentional deficits in patients with schizophrenia and in their non-psychotic first-degree relatives. *Psychiatry Res* 89: 147-159.
66. Appels MC, Sitskoorn MM, Westers P, Lems E, Kahn RS (2003): Cognitive dysfunctions in parents of schizophrenic patients parallel the deficits found in patients. *Schizophr Res* 63: 285-293.
67. Lenzenweger MF, Cornblatt BA, Putnick M (1991): Schizotypy and sustained attention. *J Abnorm Psychol* 100: 84-89.
68. Cornblatt BA, Risch NJ, Faris G, Friedman D, Erlenmeyer-Kimling L (1988): The Continuous Performance Test, identical pairs version (CPT-IP): I. New findings about sustained attention in normal families. *Psychiatry Res* 26: 223-238.
69. Cornblatt BA, Malhotra AK (2001): Impaired attention as an endophenotype for molecular genetic studies of schizophrenia. *Am J Med Genet* 105: 11-15.
70. Reitan RM (1992): *Trailmaking test. Manual for administration and scoring*. Tuscon, Reitan Neuropsychology Laboratory.
71. Lezak MD (1995): *Neurological assessment*, 3rd ed. New York: Oxford University Press.
72. Asarnow RF (1999): Neurocognitive impairments in schizophrenia: a piece of the epigenetic puzzle. *Eur Child Adolesc Psychiatry* 8 Suppl 1: I5-I8.
73. Keefe RS, Silverman JM, Roitman SE, Harvey PD, Duncan MA, Alroy D et al (1994): Performance of nonpsychotic relatives of schizophrenic patients on cognitive tests. *Psychiatry Res* 53: 1-12.
74. Harris JG, Adler LE, Young DA, Cullum CM, Rilling LM, Cicerello A et al (1996): Neuropsychological dysfunction in parents of schizophrenics. *Schizophr Res* 20: 253-260.
75. Rybakowski JK, Borkowska A (2002): Eye movement and neuropsychological studies in first-degree relatives of schizophrenic patients. *Schizophr Res* 54: 105-110.
76. Swan GE, Carmelli D (2002): Evidence for genetic mediation of executive control: a study of aging male twins. *J Gerontol B Psychol Sci Soc Sci* 57: 133-143.
77. COTAN. http://cotantestdatabase.nl/.
78. Cassens G, Inglis AK, Appelbaum PS, Gutheil TG (1990): Neuroleptics: effects on neuropsychological function in chronic schizophrenic patients. *Schizophr Bull* 16: 477-499.
79. Wolwer W, Gaebel W (2002): Impaired Trail-Making Test-B performance in patients with acute schizophrenia is related to inefficient sequencing of planning and acting. *J Psychiatr Res* 36: 407-416.
80. Erlenmeyer-Kimling L, Rock D, Roberts SA, Janal M, Kestenbaum C, Cornblatt B et al (2000): Attention, memory, and motor skills as childhood predictors of schizophrenia-related psychoses: the New York High-Risk Project. *Am J Psychiatry* 157: 1416-1422.
81. Geschwind DH, Miller BL, DeCarli C, Carmelli D (2002): Heritability of lobar brain volumes in twins supports genetic models of cerebral laterality and handedness. *Proc Natl Acad Sci U S A* 99: 3176-3181.
82. Fox PW, Hershberger SL, Bouchard TJ, Jr. (1996): Genetic and environmental contributions to the acquisition of a motor skill. *Nature* 384: 356-358.
83. Heinrichs RW, Ruttan L, Zakzanis KK, Case D (1997): Parsing schizophrenia with neurocognitive tests: evidence of stability and validity. *Brain Cogn* 35: 207-224.
84. Heinrichs RW (2001): *In Search of Madness*. New York: Oxford University Press.
85. Sitskoorn MM, Aleman A, Ebisch SJ, Appels MC, Kahn RS (2004): Cognitive deficits in relatives of patients with schizophrenia: a meta-analysis. *Schizophr Res* 71: 285-295.

86. Wechsler D (1997): *Wechsler Memory Scale,* 3rd ed. San Antonio, TX, the Psychological Corporation.
87. Gasperoni TL, Ekelund J, Huttunen M, Palmer CG, Tuulio-Henriksson A, Lonnqvist J et al (2003): Genetic linkage and association between chromosome 1q and working memory function in schizophrenia. *Am J Med Genet* 116B: 8-16.
88. Tuulio-Henriksson A, Haukka J, Partonen T, Varilo T, Paunio T, Ekelund J et al (2002): Heritability and number of quantitative trait loci of neurocognitive functions in families with schizophrenia. *Am J Med Genet* 114: 483-490.
89. Nuechterlein KH, Green MF, Kern RS, Baade LE, Barch DM, Cohen JD et al (2008): The MATRICS Consensus Cognitive Battery, part 1: test selection, reliability, and validity. *Am J Psychiatry* 165: 203-213.
90. Park S, Holzman PS, Goldman-Rakic PS (1995): Spatial working memory deficits in the relatives of schizophrenic patients. *Arch Gen Psychiatry* 52: 821-828.
91. Myles-Worsley M, Park S (2002): Spatial working memory deficits in schizophrenia patients and their first degree relatives from Palau, Micronesia. *Am J Med Genet* 114: 609-615.
92. Park S, Puschel J, Sauter BH, Rentsch M, Hell D (1999): Spatial working memory deficits and clinical symptoms in schizophrenia: a 4-month follow-up study. *Biol Psychiatry* 46: 392-400.
93. Pirkola T, Tuulio-Henriksson A, Glahn D, Kieseppa T, Haukka J, Kaprio J et al (2005): Spatial working memory function in twins with schizophrenia and bipolar disorder. *Biol Psychiatry* 58: 930-936.
94. Stinissen J, Willems PJ, Coetsier P, Hulsman WL (1970): *Dutch adaption of the Wechsler Adult Intelligent Scale.* Amsterdam, Swets & Zeitlinger.
95. Aleman A, Hijman R, de Haan EH, Kahn RS (1999): Memory impairment in schizophrenia: a meta-analysis. *Am J Psychiatry* 156: 1358-1366.
96. Cornblatt BA, Erlenmeyer-Kimling L (1985): Global attentional deviance as a marker of risk for schizophrenia: specificity and predictive validity. *J Abnorm Psychol* 94: 470-486.
97. Ando J, Ono Y, Wright MJ (2001): Genetic structure of spatial and verbal working memory. *Behav Genet* 31: 615-624.
98. Egan MF, Goldberg TE, Gscheidle T, Weirich M, Rawlings R, Hyde TM et al (2001): Relative risk for cognitive impairments in siblings of patients with schizophrenia. *Biol Psychiatry* 50: 98-107.
99. Delis DC, Kramer JH, Fridlund AJ, Kaplan E (1990): A cognitive science approach to neuropsyhcological assessment. In McReynolds P, Rosen JC, Chelune G, editors. *Advances in psychological assessment: Vol. 7.* New York: Plenum.
100. Benton AL (1968): Differential behavioral effects in frontal lobe disease. *Neuropsychologia* 6: 56-60.
101. Kremen WS, Seidman LJ, Pepple JR, Lyons MJ, Tsuang MT, Faraone SV (1994): Neuropsychological risk indicators for schizophrenia: a review of family studies. *Schizophr Bull* 20: 103-119.
102. Chen YL, Chen YH, Lieh-Mak F (2000): Semantic verbal fluency deficit as a familial trait marker in schizophrenia. *Psychiatry Res* 95: 133-148.
103. Kremen WS, Seidman LJ, Faraone SV, Tsuang MT (2003): Is there disproportionate impairment in semantic or phonemic fluency in schizophrenia? *J Int Neuropsychol Soc* 9: 79-88.
104. Benton AL, Sivan AB, Hamsher K, Varney NR, Spreen O (1994): *Contributions to neuropsychological assessment 2nd edition.* Oxford University Press, Inc.
105. Calkins ME, Gur RC, Ragland JD, Gur RE (2005): Face recognition memory deficits and visual object memory performance in patients with schizophrenia and their relatives. *Am J Psychiatry* 162: 1963-1966.
106. Silver H, Goodman C, Bilker W, Gur RC, Isakov V, Knoll G et al (2006): Impaired error monitoring contributes to face recognition deficit in schizophrenia patients. *Schizophr Res* 85: 151-161.
107. Christensen KJ, Riley BE, Heffernan KA, Love SB, Laughlin Sta Maria ME (2002): Facial recognition test in the elderly: norms, reliability and premorbid estimation. *Clin Neuropsychol* 16: 51-56.
108. Reichenberg A, Weiser M, Rapp MA, Rabinowitz J, Caspi A, Schmeidler J et al (2005): Elaboration on premorbid intellectual performance in schizophrenia: premorbid intellectual decline and risk for schizophrenia. *Arch Gen Psychiatry* 62: 1297-1304.

109. Woodberry KA, Giuliano AJ, Seidman LJ (2008): Premorbid IQ in Schizophrenia: A Meta-Analytic Review. *Am J Psychiatry* 165: 579-87.
110. Toulopoulou T, Picchioni M, Rijsdijk F, Hua-Hall M, Ettinger U, Sham P et al (2007): Substantial genetic overlap between neurocognition and schizophrenia: genetic modeling in twin samples. *Arch Gen Psychiatry* 64: 1348-1355.
111. Jensen AR (1998): *The g factor: the science of mental ability*. Westport, CT: Praeger Publishers.
112. Colom R, Rebollo I, Palacios A, Juan-Espinosa M, Kyllonen PC (2004): Working memory is (almost) perfectly predicted by g. *Intelligence* 32: 277-296.
113. Engle RW, Tuholski SW, Laughlin JE, Conway AR (1999): Working memory, short-term memory, and general fluid intelligence: a latent-variable approach. *J Exp Psychol Gen* 128: 309-331.
114. Bouchard TJ, Jr., McGue M (2003): Genetic and environmental influences on human psychological differences. *J Neurobiol* 54: 4-45.
115. Devlin B, Daniels M, Roeder K (1997): The heritability of IQ. *Nature* 388: 468-471.
116. Fisher PJ, Turic D, Williams NM, McGuffin P, Asherson P, Ball D et al (1999): DNA pooling identifies QTLs on chromosome 4 for general cognitive ability in children. *Hum Mol Genet* 8: 915-922.
117. Wechsler D (2000): *WAIS-III Nederlandstalige Bewerking. Afname en scoringshandleiding.* Lisse, Netherlands, Swets Test Publishers.
118. Blyler CR, Gold JM, Iannone VN, Buchanan RW (2000): Short form of the WAIS-III for use with patients with schizophrenia. *Schizophr Res* 46: 209-215.
119. van Os J, Jones PB (2001): Neuroticism as a risk factor for schizophrenia. *Psychol Med* 31: 1129-1134.
120. Krabbendam L, Janssen I, Bak M, Bijl RV, de Graaf R, van Os J (2002): Neuroticism and low self-esteem as risk factors for psychosis. *Soc Psychiatry Psychiatr Epidemiol* 37: 1-6.
121. Jang KL, Livesley WJ, Vernon PA (1996): Heritability of the big five personality dimensions and their facets: a twin study. *J Pers* 64: 577-591.
122. Hoekstra HA, Ormel J, de Fruyt F (1996) *NEO PI-R; NEO FFI; Big Five Persoonlijkheidsvragenlijsten; Handleiding.* Lisse, the Netherlands, Swets & Zeitlinger B.V.
123. Digman JM (1990): Personality Structure - Emergence of the 5-Factor Model. *Annual Review of Psychology* 41: 417-440.
124. Laroi F, Van der Linden M, de Fruyt F, van Os J, Aleman A (2006): Associations between delusion proneness and personality structure in non-clinical participants: comparison between young and elderly samples. *Psychopathology* 39: 218-226.
125. DeYoung CG, Peterson JB, Higgins DM (2005): Sources of openness/intellect: cognitive and neuropsychological correlates of the fifth factor of personality. *J Pers* 73: 825-858.
126. Weinberger DR, Egan MF, Bertolino A, Callicott JH, Mattay VS, Lipska BK et al (2001): Prefrontal neurons and the genetics of schizophrenia. *Biol Psychiatry* 50: 825-844.
127. Bagby RM, Bindseil KD, Schuller DR, Rector NA, Young LT, Cooke RG et al (1997): Relationship between the five-factor model of personality and unipolar, bipolar and schizophrenic patients. *Psychiatry Res* 70: 83-94.
128. Camisa KM, Bockbrader MA, Lysaker P, Rae LL, Brenner CA, O'Donnell BF (2005): Personality traits in schizophrenia and related personality disorders. *Psychiatry Res* 133: 23-33.
129. Ross SR, Lutz CJ, Bailley SE (2002): Positive and negative symptoms of schizotypy and the Five-factor model: a domain and facet level analysis. *J Pers Assess* 79: 53-72.
130. Tsuang MT, Stone WS, Faraone SV (1999): The genetics of schizophrenia. *Curr Psychiatry Rep* 1: 20-24.
131. Fanous A, Gardner C, Walsh D, Kendler KS (2001): Relationship between positive and negative symptoms of schizophrenia and schizotypal symptoms in nonpsychotic relatives. *Arch Gen Psychiatry* 58: 669-673.
132. Asarnow RF, Nuechterlein KH, Asamen J, Fogelson D, Subotnik KL, Zaucha K et al (2002): Neurocognitive functioning and schizophrenia spectrum disorders can be independent expressions of familial liability for schizophrenia in community control children: the UCLA family study. *Schizophr Res* 54: 111-120.
133. Tsuang MT, Stone WS, Tarbox SI, Faraone SV (2002): An integration of schizophrenia with schizotypy: identification of schizotaxia and implications for research on treatment and prevention. *Schizophr Res* 54: 169-175.

134. Vollema MG, Sitskoorn MM, Appels MC, Kahn RS (2002): Does the Schizotypal Personality Questionnaire reflect the biological-genetic vulnerability to schizophrenia? *Schizophr Res* 54: 39-45.
135. Vogel F, Schalt E, Kruger J, Propping P, Lehnert KF (1979): The electroencephalogram (EEG) as a research tool in human behavior genetics: psychological examinations in healthy males with various inherited EEG variants. I. Rationale of the study. Material. Methods. Heritability of test parameters. *Hum Genet* 47: 1-45.
136. Uhlhaas PJ, Haenschel C, Nikolic D, Singer W (2008): The role of oscillations and synchrony in cortical networks and their putative relevance for the pathophysiology of schizophrenia. *Schizophr Bull* 34: 927-943.
137. Doege K, Bates AT, White TP, Das D, Boks MP, Liddle PF (2009): Reduced event-related low frequency EEG activity in schizophrenia during an auditory oddball task. *Psychophysiology* 46: 566-577.
138. Boutros NN, Arfken C, Galderisi S, Warrick J, Pratt G, Iacono W (2008): The status of spectral EEG abnormality as a diagnostic test for schizophrenia. *Schizophr Res* 99: 225-237.
139. Kirino E (2004): Correlation between P300 and EEG rhythm in schizophrenia. *Clin EEG Neurosci* 35: 137-146.
140. Vogel F (1970): The genetic basis of the normal human electroencephalogram (EEG). *Humangenetik* 10: 91-114.
141. Zietsch BP, Hansen JL, Hansell NK, Geffen GM, Martin NG, Wright MJ (2007): Common and specific genetic influences on EEG power bands delta, theta, alpha, and beta. *Biol Psychol* 75: 154-164.
142. Stassen HH, Coppola R, Gottesman II, Torrey EF, Kuny S, Rickler KC et al (1999): EEG differences in monozygotic twins discordant and concordant for schizophrenia. *Psychophysiology* 36: 109-117.
143. van Beijsterveldt CE, van Baal GC (2002): Twin and family studies of the human electroencephalogram: a review and a meta-analysis. *Biol Psychol* 61: 111-138.
144. Lund TR, Sponheim SR, Iacono WG, Clementz BA (1995): Internal consistency reliability of resting EEG power spectra in schizophrenic and normal subjects. *Psychophysiology* 32: 66-71.
145. Pollock VE, Schneider LS, Lyness SA (1991): Reliability of topographic quantitative EEG amplitude in healthy late-middle-aged and elderly subjects. *Electroencephalogr Clin Neurophysiol* 79: 20-26.
146. Salinsky MC, Oken BS, Morehead L (1991): Test-retest reliability in EEG frequency analysis. *Electroencephalogr Clin Neurophysiol* 79: 382-392.
147. Lifshitz K, Lee KL, Susswein S (1987): Long-term replicability of EEG spectra and auditory evoked potentials in schizophrenic and normal subjects. *Neuropsychobiology* 18: 205-211.
148. Itil TM (1977): Qualitative and quantitative EEG findings in schizophrenia. *Schizophr Bull* 3: 61-79.
149. Alfimova M, Uvarova L (2003): Cognitive peculiarities in relatives of schizophrenic and schizoaffective patients: heritability and resting EEG-correlates. *Int J Psychophysiol* 49: 201-216.
150. Venables NC, Bernat EM, Sponheim SR (2008): Genetic and Disorder-Specific Aspects of Resting State EEG Abnormalities in Schizophrenia. *Schizophr Bull* .
151. Green AE, Munafo MR, DeYoung CG, Fossella JA, Fan J, Gray JR (2008): Using genetic data in cognitive neuroscience: from growing pains to genuine insights. *Nat Rev Neurosci* 9: 710-720.
152. Meyer-Lindenberg A, Weinberger DR (2006): Intermediate phenotypes and genetic mechanisms of psychiatric disorders. *Nat Rev Neurosci* 7: 818-827.
153. Faraone SV, Seidman LJ, Kremen WS, Toomey R, Pepple JR, Tsuang MT (2000): Neuropsychologic functioning among the nonpsychotic relatives of schizophrenic patients: the effect of genetic loading. *Biol Psychiatry* 48: 120-126.
154. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE et al (2001): Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A* 98: 6917-6922.
155. Flint J, Munafo MR (2006): The endophenotype concept in psychiatric genetics. *Psychol Med* : 1-18.
156. Coon H, Plaetke R, Holik J, Hoff M, Myles-Worsley M, Waldo M et al (1993): Use of a neurophysiological trait in linkage analysis of schizophrenia. *Biol Psychiatry* 34: 277-289.

157. Almasy L, Porjesz B, Blangero J, Goate A, Edenberg HJ, Chorlian DB et al (2001): Genetics of event-related brain potentials in response to a semantic priming paradigm in families with a history of alcoholism. *Am J Hum Genet* 68: 128-135.
158. Bautista JF, Quade SR, Parrado AR, Goddard KA (2005): Linkage analysis of alcoholism-related electrophysiological phenotypes: genome scans with microsatellites compared to single-nucleotide polymorphisms. *BMC Genet* 6 Suppl 1: S156.
159. Begleiter H, Porjesz B, Reich T, Edenberg HJ, Goate A, Blangero J et al (1998): Quantitative trait loci analysis of human event-related brain potentials: P3 voltage. *Electroencephalogr Clin Neurophysiol* 108: 244-250.
160. Dick DM, Aliev F, Bierut L, Goate A, Rice J, Hinrichs A et al (2006): Linkage analyses of IQ in the collaborative study on the genetics of alcoholism (COGA) sample. *Behav Genet* 36: 77-86.
161. Jones KA, Porjesz B, Almasy L, Bierut L, Goate A, Wang JC et al (2004): Linkage and linkage disequilibrium of evoked EEG oscillations with CHRM2 receptor gene polymorphisms: implications for human brain dynamics and cognition. *Int J Psychophysiol* 53: 75-90.
162. Porjesz B, Begleiter H, Wang K, Almasy L, Chorlian DB, Stimus AT et al (2002): Linkage and linkage disequilibrium mapping of ERP and EEG phenotypes. *Biol Psychol* 61: 229-248.
163. Porjesz B, Almasy L, Edenberg HJ, Wang K, Chorlian DB, Foroud T et al (2002): Linkage disequilibrium between the beta frequency of the human EEG and a GABAA receptor gene locus. *Proc Natl Acad Sci U S A* 99: 3729-3733.
164. Luciano M, Wright MJ, Duffy DL, Wainwright MA, Zhu G, Evans DM et al (2006): Genome-wide scan of IQ finds significant linkage to a quantitative trait locus on 2q. *Behav Genet* 36: 45-55.
165. Posthuma D, Luciano M, Geus EJ, Wright MJ, Slagboom PE, Montgomery GW et al (2005): A genomewide scan for intelligence identifies quantitative trait loci on 2q and 6p. *Am J Hum Genet* 77: 318-326.
166. Fullerton J, Cubin M, Tiwari H, Wang C, Bomhra A, Davidson S et al (2003): Linkage analysis of extremely discordant and concordant sibling pairs identifies quantitative-trait loci that influence variation in the human personality trait neuroticism. *Am J Hum Genet* 72: 879-890.
167. Kuo PH, Neale MC, Riley BP, Patterson DG, Walsh D, Prescott CA et al (2007): A genome-wide linkage analysis for the personality trait neuroticism in the Irish affected sib-pair study of alcohol dependence. *Am J Med Genet B Neuropsychiatr Genet* 144B: 463-468.
168. Nash MW, Huezo-Diaz P, Williamson RJ, Sterne A, Purcell S, Hoda F et al (2004): Genome-wide linkage analysis of a composite index of neuroticism and mood-related scales in extreme selected sibships. *Hum Mol Genet* 13: 2173-2182.
169. Neale BM, Sullivan PF, Kendler KS (2005): A genome scan of neuroticism in nicotine dependent smokers. *Am J Med Genet B Neuropsychiatr Genet* 132B: 65-69.
170. Antonarakis SE, Blouin JL, Pulver AE, Wolyniec P, Lasseter VK, Nestadt G et al (1995): Schizophrenia susceptibility and chromosome 6p24-22. *Nat Genet* 11: 235-236.
171. Bailer U, Leisch F, Meszaros K, Lenzinger E, Willinger U, Strobl R et al (2000): Genome scan for susceptibility loci for schizophrenia. *Neuropsychobiology* 42: 175-182.
172. Ekelund J, Lichtermann D, Hovatta I, Ellonen P, Suvisaari J, Terwilliger JD et al (2000): Genome-wide scan for schizophrenia in the Finnish population: evidence for a locus on chromosome 7q22. *Hum Mol Genet* 9: 1049-1057.
173. Hennah W, Tomppo L, Hiekkalinna T, Palo OM, Kilpinen H, Ekelund J et al (2007): Families with the risk allele of DISC1 reveal a link between schizophrenia and another component of the same molecular pathway, NDE1. *Hum Mol Genet* 16: 453-462.
174. Hovatta I, Lichtermann D, Juvonen H, Suvisaari J, Terwilliger JD, Arajarvi R et al (1998): Linkage analysis of putative schizophrenia gene candidate regions on chromosomes 3p, 5q, 6p, 8p, 20p and 22q in a population-based sampled Finnish family set. *Mol Psychiatry* 3: 452-457.
175. Klei L, Bacanu SA, Myles-Worsley M, Galke B, Xie W, Tiobech J et al (2005): Linkage analysis of a completely ascertained sample of familial schizophrenics and bipolars from Palau, Micronesia. *Hum Genet* 117: 349-356.
176. Lerer B, Segman RH, Hamdan A, Kanyas K, Karni O, Kohn Y et al (2003): Genome scan of Arab Israeli families maps a schizophrenia susceptibility gene to chromosome 6q23 and supports a locus at chromosome 10q24. *Mol Psychiatry* 8: 488-498.

177. Lindholm E, Ekholm B, Balciuniene J, Johansson G, Castensson A, Koisti M et al (1999): Linkage analysis of a large Swedish kindred provides further support for a susceptibility locus for schizophrenia on chromosome 6p23. *Am J Med Genet* 88: 369-377.
178. Maziade M, Roy MA, Rouillard E, Bissonnette L, Fournier JP, Roy A et al (2001): A search for specific and common susceptibility loci for schizophrenia and bipolar disorder: a linkage study in 13 target chromosomes. *Mol Psychiatry* 6: 684-693.
179. Paunio T, Ekelund J, Varilo T, Parker A, Hovatta I, Turunen JA et al (2001): Genome-wide scan in a nationwide study sample of schizophrenia families in Finland reveals susceptibility loci on chromosomes 2q and 5q. *Hum Mol Genet* 10: 3037-3048.
180. Schwab SG, Hallmayer J, Albus M, Lerer B, Eckstein GN, Borrmann M et al (2000): A genome-wide autosomal screen for schizophrenia susceptibility loci in 71 families with affected siblings: support for loci on chromosome 10p and 6. *Mol Psychiatry* 5: 638-649.
181. Straub RE, MacLean CJ, Ma Y, Webb BT, Myakishev MV, Harris-Kerr C et al (2002): Genome-wide scans of three independent sets of 90 Irish multiplex schizophrenia families and follow-up of selected regions in all families provides evidence for multiple susceptibility genes. *Mol Psychiatry* 7: 542-559.
182. Wedenoja J, Loukola A, Tuulio-Henriksson A, Paunio T, Ekelund J, Silander K et al (2008): Replication of linkage on chromosome 7q22 and association of the regional Reelin gene with working memory in schizophrenia families. *Mol Psychiatry* 13: 673-684.
183. Wijsman EM, Rosenthal EA, Hall D, Blundell ML, Sobin C, Heath SC et al (2003): Genome-wide scan in a large complex pedigree with predominantly male schizophrenics from the island of Kosrae: evidence for linkage to chromosome 2q. *Mol Psychiatry* 8: 695-705, 643.
184. Paunio T, Tuulio-Henriksson A, Hiekkalinna T, Perola M, Varilo T, Partonen T et al (2004): Search for cognitive trait components of schizophrenia reveals a locus for verbal learning and memory on 4q and for visual working memory on 2q. *Hum Mol Genet* 13: 1693-1702.
185. Hansell NK, Medland SE, Ferreira MA, Geffen GM, Zhu G, Montgomery GW et al (2006): Linkage analyses of event-related potential slow wave phenotypes recorded in a working memory task. *Behav Genet* 36: 29-44.
186. Hennah W, Tuulio-Henriksson A, Paunio T, Ekelund J, Varilo T, Partonen T et al (2005): A haplotype within the DISC1 gene is associated with visual memory functions in families with a high density of schizophrenia. *Mol Psychiatry* 10: 1097-1103.
187. Ferguson SC, Deary IJ, Perros P, Evans JC, Ellard S, Hattersley AT et al (2003): Apolipoprotein-e influences aspects of intellectual ability in type 1 diabetes. *Diabetes* 52: 145-148.
188. Goldberg TE, Egan MF, Gscheidle T, Coppola R, Weickert T, Kolachana BS et al (2003): Executive subprocesses in working memory: relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. *Arch Gen Psychiatry* 60: 889-896.
189. Harris SE, Fox H, Wright AF, Hayward C, Starr JM, Whalley LJ et al (2006): The brain-derived neurotrophic factor Val66Met polymorphism is associated with age-related change in reasoning skills. *Mol Psychiatry* 11: 505-513.
190. Savitz J, Solms M, Ramesar R (2006): The molecular genetics of cognition: dopamine, COMT and BDNF. *Genes Brain Behav* 5: 311-328.
191. Esslinger C, Walter H, Kirsch P, Erk S, Schnell K, Arnold C et al (2009): Neural mechanisms of a genome-wide supported psychosis variant. *Science* 324: 605.
192. Huffaker SJ, Chen J, Nicodemus KK, Sambataro F, Yang F, Mattay V et al (2009): A primate-specific, brain isoform of KCNH2 affects cortical physiology, cognition, neuronal repolarization and risk of schizophrenia. *Nat Med* 15: 509-518.
193. Tan HY, Chen Q, Sust S, Buckholtz JW, Meyers JD, Egan MF et al (2007): Epistasis between catechol-O-methyltransferase and type II metabotropic glutamate receptor 3 genes on working memory brain function. *Proc Natl Acad Sci U S A* 104: 12536-12541.
194. Butcher LM, Davis OS, Craig IW, Plomin R (2008): Genome-wide quantitative trait locus association scan of general cognitive ability using pooled DNA and 500K single nucleotide polymorphism microarrays. *Genes Brain Behav* 7: 435-446.

Chapter 2

Finding Suitable Phenotypes for Genetic Studies of Schizophrenia: Heritability and Segregation Analysis

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Abstract

Background: Schizophrenia is a highly heritable and complex disorder. Multiple genes are likely to be involved, complicating genetic research into the aetiology of this disorder. Intermediate phenotypes or endophenotypes may facilitate genetic research if they display a simpler mode of transmission than schizophrenia itself, i.e., if they reflect more closely the underlying genetic effects.

Methods: Twenty-five multigenerational families with multiple members affected with schizophrenia (180 subjects) were administered an extensive neuropsychological, psychophysiological and personality test battery. Familial correlations were calculated to select heritable traits. Subsequent heritability analysis followed by commingling and segregation analysis was performed to unravel the pattern of transmission and to estimate heritability.

Results: Five traits, including sensorimotor gating, openness, verbal fluency, early visual perception, and spatial working memory, showed moderate familial correlations. Heritability estimates for these traits ranged from 37% to 54%. A major gene model resembling dominant transmission was found for both sensorimotor gating and openness. Verbal fluency, early visual perception, and spatial working memory may be accounted for by polygenic, multifactorial, or environmental effects.

Conclusions: Only 2 of 13 candidate endophenotypes showed a simple mode of transmission useful for successful application in molecular genetic research: sensorimotor gating and openness. To our knowledge, this is the first study to investigate the pattern of transmission for these traits.

Introduction

Although schizophrenia is highly heritable (~80%)[1] gene finding studies have reported conflicting results.[2] This may be due to the likelihood that schizophrenia is caused by multiple genes interacting with each other and with environmental factors, leading to a complex mode of transmission.[3,4] To overcome difficulties such as genetic and phenotypic heterogeneity, the use of endophenotypes may be a promising alternative strategy. Endophenotypes may increase power in quantitative gene mapping by their putative simpler mode of transmission, quantitative nature, and potential to identify the unaffected but potentially gene-carrying relatives.[5,6] Indeed, several studies reported stronger linkage findings for the endophenotypic trait than for the clinical diagnosis.[7-9]

Meta-analyses have reported abnormalities on numerous characteristics of schizophrenia to accumulate among the unaffected relatives of patients with schizophrenia as compared to the general population,[10,11] suggesting a potentially shared genetic aetiology between the characteristics and schizophrenia. This is supported by an increasing number of studies reporting substantial genetic contributions to (some of) these candidate endophenotypes.[12-15] However, heritability estimates do not provide information on the mode of transmission, i.e., whether the trait is influenced by a single major gene, a small set of genes, or complex interactions. Clearly, using endophenotypes with a complex mode of transmission in genetic research may not provide advantages over the schizophrenia phenotype itself. Ideally, endophenotypes show a mode of inheritance that may be caused by few major genetic variants, and therefore may provide a simpler means to identify schizophrenia- predisposing variants.

Relatively few studies have examined the mode of transmission of a limited number of schizophrenia-related endophenotypes: oculomotor dysfunction,[16,17] P50 ratio,[18] P300 latency [19] and spatial working memory, verbal declarative memory, and verbal and visual ability.[20] Some of these phenotypes have been mapped to specific genetic loci: 6p21-23,[21-23] 15q14,[24,25] 22q11-q12,[26] 4q21, and suggestive evidence to regions on 1q, 2q, 8q, 9p, 10p and 15q.[8] Interestingly, some of these regions may overlap with schizophrenia loci.[27] The strategy of linking endophenotypes with a simple mode of transmission to specific genetic loci has thus been successful. We have therefore studied both heritability and mode of transmission of a selected number of promising (additional) endophenotypes. We selected 13 neuropsychological, psychophysiological and personality candidate endophenotypes based on previous studies in unaffected relatives and high-risk populations, as well as on the basis of reliability, stability and heritability estimates if available. First, we calculated familial correlations to select potentially heritable traits. Subsequent heritability analysis followed by commingling and segregation analyses were performed to esti-

mate heritability and to unravel the pattern of transmission. Ultimately, endophenotypes showing a clear pattern of inheritance may result in finding new genes and biological pathways involved in schizophrenia.

Methods and Materials

Participants

Twenty-five multiplex multigenerational pedigrees of Dutch origin were recruited from the base population through a schizophrenia family member association and through an advertisement in a Dutch daily newspaper. Each pedigree comprised at least two members with a schizophrenia or schizoaffective disorder diagnosis based on the Family Interview for Genetic Studies (FIGS),[28] and at least one member's diagnosis was confirmed by interview. Exclusion criteria for patients and family members were: severe medical or neurological illness; history of closed-head injury; loss of consciousness longer than 30 minutes; history of alcohol abuse within last 6 months; diseases of the central nervous system and history of CVAs, dementia or delirium; aged under 16; or IQ under 70. Specific exclusion criteria for separate tests are given in the relevant method sections. For the personality questionnaire no exclusion criteria applied. Written informed consent was obtained from all participants. The study was approved by the Medical Ethics Committee of the University Medical Center (UMC) Utrecht.

Diagnostic assessment

Each family was screened using the FIGS conducted by telephone with a family member. Patients were diagnosed by DSM-IV criteria based on the Comprehensive Assessment of Symptoms and History (CASH), a semi-structured diagnostic interview,[29] and by retrieving medical records. A master's level clinician (M.F.A.) conducted the interview, and discussed the results with a psychiatrist (J.-P.S.). Lifetime experienced psychiatric episodes in relatives were assessed (by M.F.A.) using the Mini-International Neuropsychiatric Interview-Structured (MINIPlus), a clinical interview for DSM-IV Axis I disorders.[30]

Cognitive measures

An extensive neuropsychological and psychophysiological test battery, conducted in the same order for every participant at the UMC Utrecht, lasted about 4 hours, excluding a sufficient number of breaks. Personality questionnaires were completed beforehand. We selected 13 measures on the basis of fulfilling as many criteria as possible for candidate endophenotypes.[5,31] (Table 1;

Table 1 Parent-offspring and sib-sib correlations of all endophenotypes

Test	Concept	n_{PO}	$\rho_{PO} \pm SE$	n_{SS}	$\rho_{SS} \pm SE$
PPI	Sensorimotor gating	78	0.38 ± 0.10	98	0.01 ± 0.11
Openness	Personality: Openness to experience	115	0.32 ± 0.13	138	0.33 ± 0.13
Backward Masking					
location	Early visual perception, global	75	0.22 ± 0.15	81	0.23 ± 0.14
identification	Early visual perception, local	74	0.04 ± 0.10	83	-0.10 ± 0.10
Spatial span					
forward	Spatial attention	98	0.22 ± 0.10	112	0.14 ± 0.12
total	Spatial working memory	98	0.21 ± 0.12	112	0.22 ± 0.13
backward	Spatial working memory	98	0.16 ± 0.13	112	0.21 ± 0.13
Verbal Fluency					
categories	Semantic fluency	94	0.17 ± 0.10	106	0.34 ± 0.14
letters	Phonemic fluency	87	0.05 ± 0.14	109	0.29 ± 0.13
CPT-IP	Continuous performance				
logB verbal	Conservative response bias (verbal)	81	0.16 ± 0.10	94	-0.01 ± 0.11
d' verbal	Level of attention (verbal)	81	-0.06 ± 0.11	94	-0.08 ± 0.10
d' spatial	Level of attention (spatial)	81	0.03 ± 0.11	94	-0.03 ± 0.10
logB spatial	Conservative response bias (spatial)	81	-0.14 ± 0.11	94	0.01 ± 0.11
P50					
P50 ratio(S2/S1)	Sensory gating	76	0.13 ± 0.12	86	-0.03 ± 0.10
P50 difference (S1-S2)	Sensory gating	83	0.07 ± 0.13	102	0.00 ± 0.10
N100 (S2/S1)	Sensory gating	80	-0.10 ± 0.11	99	-0.05 ± 0.09
Neuroticism	Personality: Neuroticism	114	0.13 ± 0.08	135	-0.02 ± 0.08
Digit span					
forward	Verbal attention	92	0.13 ± 0.10	108	0.03 ± 0.10
total	Verbal working memory	92	0.10 ± 0.10	108	-0.02 ± 0.09
backward	Verbal working memory	92	-0.01 ± 0.09	108	-0.13 ± 0.07
CVLT					
immediate recall	Immediate verbal memory	91	0.12 ± 0.11	108	0.09 ± 0.11
short delay recall	Short delay verbal memory	92	0.11 ± 0.13	108	0.09 ± 0.11
delayed free recall	Delayed verbal memory	92	-0.02 ± 0.13	108	0.06 ± 0.11
Facial Recognition Test	Facial recognition	95	0.09 ± 0.13	108	0.16 ± 0.13
Purdue pegboard	Psychomotor dexterity	85	0.04 ± 0.11	91	0.23 ± 0.14
Trail Making (B-A)	Set shifting	97	-0.00 ± 0.12	109	0.04 ± 0.10

Note: italics indicate variables of the test. Bold type are the measures selected for further analyses (see text); for each selected measure, we used the variable with the highest PO and SS correlations.

Abbreviations: CVLT: Californian Verbal Learning Task; CPT-IP: Continuous performance Test – Identical Pairs; n_{PO}: number of parent-offspring pairs; n_{SS}: number of sib-sib pairs; ρ_{PO}: parent-offspring correlation; ρ_{SS}: sib-sib correlation; PPI: Prepulse Inhibition; S1: stimulus 1; S2: stimulus 2; SE: standard error.

Supplement 1) The following five measures were selected on the basis of familial correlation (see below) and are described here in detail.

Sensorimotor gating Prepulse inhibition (PPI) of the startle response is a measure of sensorimotor gating, with a high inhibition score indicating better gating. Schizophrenia patients show less gating, i.e., lower inhibition scores, than controls.[32] The assessment of PPI is described in Supplement 1. Briefly, a startle is elicited by a 107 dB burst of white noise of 30 msec, preceded by a 25 msec prepulse stimulus of 87 dB white noise in 50% of the trials to induce PPI. Prepulse inhibition is defined as the percent reduction in startle magnitude in the presence of the prepulse compared with the magnitude in the absence of the prepulse.

Openness to experience Openness to experience (openness) is often characterised as cognitive flexibility or exploration. Patients with schizophrenia score lower than controls on openness.[33,34] Openness was measured as part of the Neuroticism Extroversion Openness - Five Factor Inventory (NEO-FFI),[35,36] a 60-item questionnaire that measures the major dimensions of personality, the Big Five.

Verbal fluency Category Fluency[37] is regarded as an executive task that gives an indication of verbal fluency. Meta-analyses show lower performance on the task in schizophrenic patients than in controls.[38,39] Scores equal the number of words within the categories animals and professions generated in 60 seconds.

Early visual perception Backward masking is a test of early visual information processing. The location condition taps activity of transient visual channels. In schizophrenia patients performance is lower than in controls.[40] We used the computerised visual masking battery by Green et al.[41] Procedures are described in Supplement 1. Briefly, the backward masking paradigm involves a target (a square with an opening at one side) that is presented for 13.3 msec, followed by a mask that consists of boxes occupying all possible target locations. The stimulus onset asynchrony (SOA) varied from 0 to 80 msec. Participants indicated where the target appeared. Scores were calculated as the arc sinus transformations of the mean percentage correct answers on the 6 'location' trials with incrementing SOAs.

Spatial working memory The Spatial Span subtest of the Wechsler Memory Scale-III (WMS-III)[42] is a test of spatial working memory and attention. Patients with schizophrenia show deficits on spatial working memory.[43] The test involves increasing sequences of three dimensional blocks pointed at by the experimenter that are repeated by the participant in the same order (forwards) or in reverse order (backwards condition). Scores equal the sum of correct repetitions in both conditions.

Data analysis

First, we performed parent-offspring (PO) and sib-sib (SS) correlations using the Familial Correlations (FCOR) module of the Statistical Analyses for Genetic Epidemiology (S.A.G.E.; Case Western Reserve University, Cleveland, Ohio) package[44] to provide a robust indication of familiarity in the selection of endophenotypes for further analyses. Both PO and SS pairs share half of the additive genetic variation, while SS pairs also share a quarter of dominance genetic variation and common environment. To capture the most likely heritable traits for further analysis, we selected endophenotypes with a PO correlation above 0.2, or higher than 0.1 when SS correlation was above 0.2.

Heritability analysis was performed using the variance component-based program SOLAR (Southwest Foundation for Biomedical Research, San Antonio, Texas).[45] It measures the narrow sense heritability defined as the phenotypic variance explained by additive genetic factors. Components of variance were estimated by maximum likelihood, including variation caused by the covariates age and sex in a multistep procedure. The significance of the heritability estimate was computed by comparing the polygenic model with the significant covariates to a sporadic model that had the genetic component removed.

Commingling analysis (using S.A.G.E.)[44] provides guidance in choosing initial parameters for segregation analysis. It is used for metric traits that are either polygenic or multifactorial, fits and compares mixtures of up to three normal distributions and performs Box and Cox transformation[46] if necessary. Data fitting a mixture distribution with two or three means, rather than one mean, is suggestive of an underlying genetic model.[47] We performed commingling analysis using both the class-D regressive model[48] and Finite Polygenic Mixed Models (FPMM)[49] to investigate both a major gene model and oligogenic models, respectively. In class-D regressive models the residual correlation among family members indicates common environment or polygenic effects that cannot be modelled by the major gene effect. We fixed spousal correlation at zero and assumed equal PO and SS correlations. In FPMM, polygenic effects are modelled by the sum of the effects of the alleles at a chosen number of diallelic loci indicated by the residual polygenic variance ($\sigma_{polygenic}$). We modelled FPMM with two or three polygenic loci with various allele frequencies. If both models resulted in the same mixture of distributions, we continued segregation analysis using the more parsimonious FPMM.

Subsequent segregation analysis (using S.A.G.E.)[44] determines if a major gene is involved in the trait's variability. It estimates maximum likelihoods of transmission probabilities and allele frequencies for various models. The transmission probability τ_i is the probability that a parent with genotype i transmits allele A to the offspring. In a Mendelian transmission model, $\tau_{AA} = 1$, $\tau_{AB} = 0.5$ and $\tau_{BB} = 0$, whereas in an environmental model all τ's are equal to the allele frequency. In the general model all parameters were set to be arbitrary and it therefore provided the best adjustment to the data. This model served as a reference model to which all other models were compared.

We used likelihood-based chi-square tests to compare distributions and transmission models. The difference in number of estimated parameters determines the number of degrees of freedom (df). When no clear discrimination between models could be made Akaike's an information criterion (AIC)[50] was used. Akaike's information criterion is defined as -2ln likelihood plus twice the number of independent parameters estimated and adjusts the likelihood estimate for the number of parameters. A better fit is indicated by a lower AIC.

The percent of trait variance attributable to the major gene was estimated using the equation $\sum_{i=1}^{3}\psi_i(\mu_i-\bar{\mu})^2$, where μ_i represents the major genotype means, i indexes the three major genotypes (1 = AA, 2 = AB, 3 = BB), ψ_i refers to the genotype frequencies, and $\bar{\mu}=\sum_{i=1}^{3}\psi_i\mu_i$ [51]. Hardy Weinberg equilibrium proportions were assumed under all models. In every analysis, we corrected for age and sex. No ascertainment correction was performed, as pedigrees were ascertained through disease status rather than task performance. Supplement 2 provides a flowchart of the study design.

Results

Sample description

A total of 35 patients and 145 relatives from 25 pedigrees (Figure 1) participated. Family size ranged from 2 to 21 relatives with a mean of 7.24 (standard deviation = 4.93), encompassing 2 to 4 generations (2 generations: 8 families, 25 participants; 3 generations:13 families, 104 participants; 4 generations: 4 families, 51 participants). No loops or consanguineous mating pairs were present. Nine patients and five relatives could not participate in endophenotypic measurement. Additionally, one patient and seven relatives fulfilled general exclusion criteria. Eight unaffected relatives scored on one psychosis item of the MINI-PLUS, involving single lifetime hallucinations, unique childhood experiences, or experiences related to alcohol use. None of these participants scored on any criterion for schizophrenia. One relative received 1 mg of risperidone to relieve symptoms of an atypical obsessive-compulsive disorder. This participant suffered from compulsive worrying and never had delusions or hallucinations. Sample descriptions are given in Table 2.

Figure 1 Two examples of pedigrees used. A black circle indicates a confirmed diagnosis of schizophrenia or schizoaffective disorder; a vertical black bar indicates a nonconfirmed diagnosis

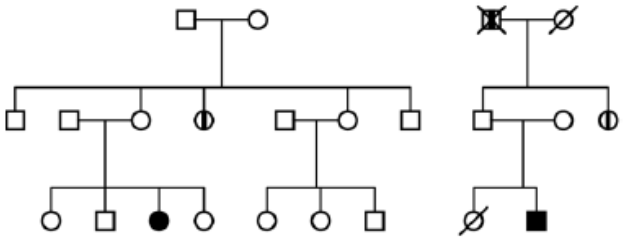


Table 2 Characteristics of the study population

	Patients (n = 35)		Relatives (n = 145)				
% female	37.14% (n = 13)		59.31% (n = 86)				
Anti-psychotics use[a]	Typical (6), atypical (24), both typical and atypical (2), none (4)		Atypical (1)[b]				
	n	M (± sd)	n	M (± sd)	t[c]	df	p
Age	35	40.74 (± 14.66)	145	48.59 (± 16.91)	-	-	-
IQ	19	96.58 (± 12.52)	114	109.02 (± 14.09)	3.62	131	<0.01
SG	18	75.82 (± 21.02)	113	61.83 (± 22.56)	-2.46	129	0.02
OP	24	38.21 (± 5.78)	139	39.34 (± 6.92)	0.76	161	0.45
VF	18	41.44 (± 9.03)	118	45.83 (± 9.27)	1.88	134	0.06
EVP	17	0.61 (± 0.28)	107	0.53 (± 0.22)	-1.44	122	0.15
SWM	21	15.24 (± 2.76)	123	16.51 (± 3.14)	1.75	142	0.08

Abbreviations: df: degrees of freedom; EVP: early visual perception; IQ: intelligence quotient; M: mean; n: number; OP: openness to experience; p: p-value; sd: standard deviation; t: t-statistic; SG: sensorimotor gating; SWM: spatial working memory; VF: verbal fluency. .

[a] In those patients that performed neuropsychological, psychophysiological tasks and/or questionnaires, antipsychotic medication use was: typical: 4, atypical: 18, both: 1, none: 3.

[b] One relative received risperidone to relieve symptoms of an atypical obsessive-compulsive disorder (see text).

[c] t-test comparing patients to relatives.

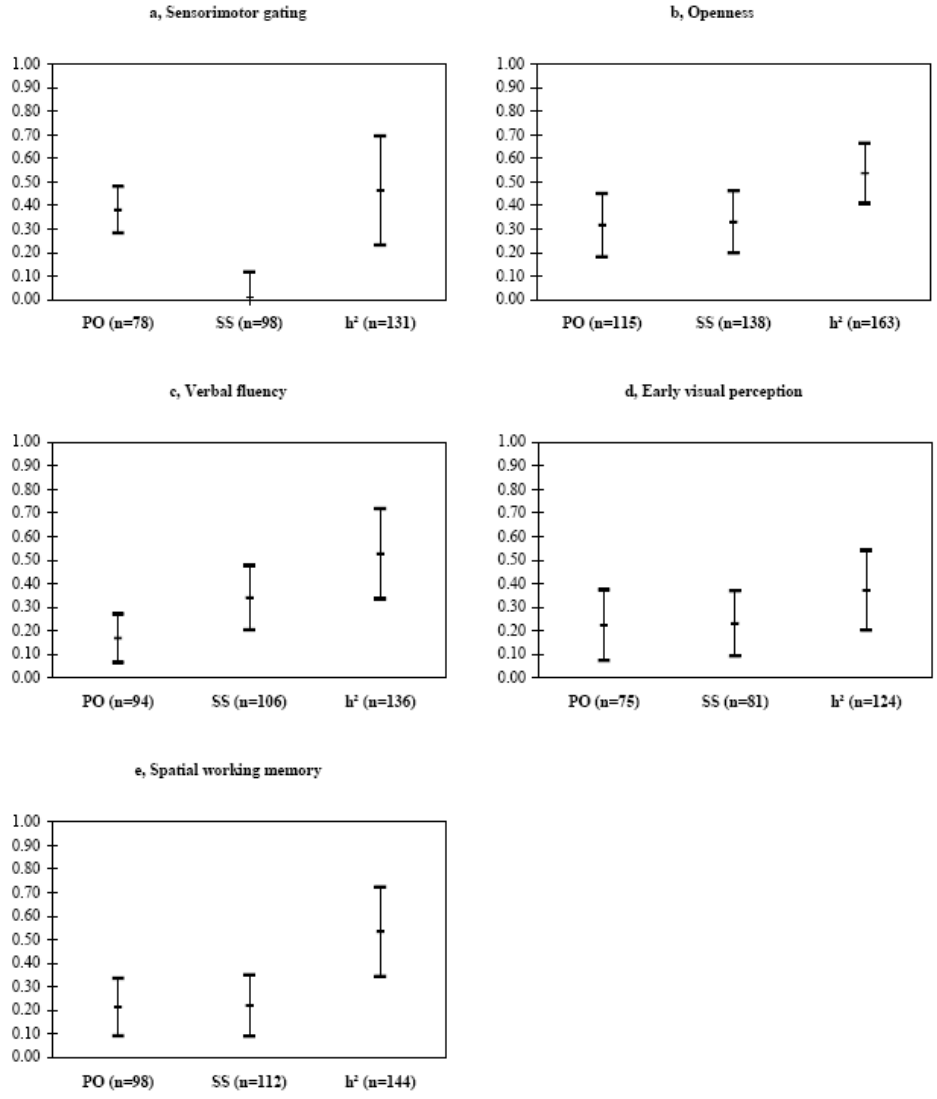
Patients and relatives differed significantly from each other in age, sex, IQ, and sensorimotor gating. Relatives performed more poorly than patients on sensorimotor gating and early visual perception. This difference is likely due to an age or a medication effect. Performance of the participants declined significantly with age for all measures except for verbal fluency. Only openness showed a significant influence of sex, with females scoring higher. The proportions of variance due to the covariates are given in Figure 2.

Inheritance

Sensorimotor gating Sensorimotor gating showed a high PO correlation of 0.38 ± 0.10 (= standard error) and no SS correlation (-0.01 ± 0.11; Table 1; Figure 2A). Differential transmission of genetic risk variants, X-linked transmission, or shared environment may account for this discrepancy. Despite the minimal SS correlation, heritability was high (0.46 ± 0.23; Figure 2A). Correspondingly, commingling analysis showed that the class-D two means distribution model had the lowest AIC score, and thus the best fit, compared to the one or three means distribution (Table 3). Similarly, the FPMM two means distribution fitted better compared to the one mean (signifi-

cantly) and three means distribution. We continued segregation analysis using the more parsimonious FPMM. The no-transmission model, i.e., environmental model, was rejected ($\chi^2 = 6.57$ (2 df), $p = 0.04$ compared with general reference model; Table 4; Table 1 in Supplement 3). The

Figure 2 Parent-offspring correlation (PO), sib-sib correlation (SS) and heritability (h^2) ± standard errors for the selected endophenotypes (a-e).



Covariates (age, sex) significant at the 0.1 level were included in the models of the heritability analyses. Proportion of variance due to all final covariates: sensorimotor gating-age: 0.05; openness-age and sex: 0.06; verbal fluency: none; early visual perception-age: 0.45; spatial working memory-age: 0.20.

n: number of pairs or participants; PO: parent-offspring correlation; SS: sib-sib correlation.

Mendelian model was not rejected by the reference model (χ^2 = 5.22 (2), p = 0.07) and gave the best fit (i.e., lowest AIC of 358.87 versus 360.14 or higher for the other transmission models). When τ was set to be free, the other models converged to a Mendelian model (Table 1 in Supplement 3), supporting a major gene effect. The Mendelian model showed dominant transmission that was associated with higher scores and thus better performance (10.59 ± 0.14, of A-allele carriers versus 9.18 ± 0.32, of the BB homozygotes; Table 5). Polygenic variance converged to zero, indicating trivial residual polygenic effects. The proportion of the variance explained by the Mendelian model was 47%, which corresponds to the heritability estimate of 0.46, suggesting it may explain most of the genetic variance of this trait.

Table 3 Commingling analyses: model fit of class-D regressive models and Finite Polygenic Mixed Models for selected endophenotypes

							Distribution comparison					
							Versus two means			Versus three means		
Trait	Model	L	d	-2lnL	AIC	N	χ^2	df	p	χ^2	df	p
SG	Class-D	-	One	351.55	359.55	4	5.46	2	0.07	6.06	3	0.11
			Two	346.09	358.09	6				0.60	1	0.44
			Three	345.49	359.49	7						
	FPMM	2	One	358.06	364.06	3	7.84	2	0.02	7.93	3	0.05
			Two	350.22	360.22	5				0.09	1	0.76
			Three	350.13	362.13	6						
OP	Class-D	-	One	458.65	466.65	4	23.01	2	<0.01	23.29	3	<0.01
			Two	435.64	447.64	6				0.28	1	0.60
			Three	435.36	449.36	7						
	FPMM	2	One	457.48	463.48	3	2.58	2	0.28	5.37	3	0.15
			Two	454.90	464.90	5				2.78	1	0.10
			Three	452.12	464.12	6						
VF	Class-D	-	One	372.96	380.96	4	2.40	2	0.30	6.53	2	0.04
			Two	370.55	382.55	6				4.13	0	nc
			Three	366.43	378.43	6						
	FPMM	2	One	382.87	388.87	3	4.31	3	0.23	10.10	4	0.04
			Two	378.56	390.56	6				5.80	1	0.02
			Three	372.77	386.77	7						
EVP	Class-D	-	One	342.46	350.46	4	6.13	2	0.05	6.92	3	0.07
			Two	336.33	348.33	6				0.80	1	0.37
			Three	335.54	349.54	7						
	FPMM	3	One	348.70	354.70	3	2.16	3	0.54	2.30	4	0.68
			Two	346.54	358.54	6				0.14	1	0.71
			Three	346.39	360.39	7						
SWM	Class-D	-	One	396.52	404.52	4	2.26	2	0.32	2.26	3	0.52
			Two	394.27	406.27	6				0.00	1	1.00
			Three	394.27	408.27	7						
	FPMM	3	One	402.95	410.95	4	0.66	2	0.72	0.97	3	0.81
			Two	402.29	414.29	6				0.31	1	0.58
			Three	401.98	415.98	7						

Abbreviations: AIC: An Information Criterion; χ^2: chi square statistic; d: number of distributions; df: degrees of freedom; EVP: early visual perception; FPMM: Finite Polygenic Mixed Models; L: number of loci modelled in FPMM; -2lnL: -2log likelihood; N: number of estimated parameters; nc: not comparable; OP: openness to experience; SG: sensorimotor gating; SWM: spatial working memory; VF: verbal fluency.

Table 4 Segregation analyses: Akaike's An Information criterion (AIC) model fit: likelihood estimates

Trait	Model	L	d	Hom no transmission	Hom mendelian	Hom general	τ_{AB}-free	General (Ref.)
SG	FPMM	2	Two	360.22[a]	358.87	360.14	360.51[a]	357.65[b]
OP	Class-D	-	Two	447.64[a]	444.12	443.75[b]	443.80[b]	445.67[b]
VF	FPMM	2	Three	392.55[a]	381.06[a,b]	382.93[a,b]	373.58[b]	375.47[b]
EVP	Class-D	-	Two	348.33	347.49	350.56	347.07	350.56

Abbreviations: d: number of distributions; EVP: early visual perception; FPMM: Finite Polygenic Mixed Models; Hom: homogeneous; L: number of loci fitted in FPMM; OP: openness to experience; Ref.: reference model; SG: sensorimotor gating; VF: verbal fluency..

[a] The corresponding -2likelyhood is significantly different from the reference model at $p < 0.05$.

[b] The corresponding -2likelyhood is significantly different from the no transmission model at $p < 0.05$.

Openness The equivalent PO (0.32 ± 0.13) and SS correlations (0.33 ± 0.13) for openness were compatible with its heritability estimate in the high range (0.54 ± 0.13; Figure 2B). Class-D commingling analysis fitted a two means distribution, whereas the FPMM did not show a mixture of distributions (Table 3). In subsequent segregation analysis of the class-D model, the no-transmission model was rejected ($\chi^2 = 5.96$ (2), $p = 0.05$, Tables 4; Table 2 in Supplement 3)), and the Mendelian model revealing a recessive pattern was not rejected by the reference model ($\chi^2 = 2.45$ (2), $p = 0.29$). The τ_{AB}-free and the homogeneous general models showed a slightly better fit (with similar AIC's of 443.80 and 443.75, respectively) than the Mendelian model (444.12) and were significantly better than the no-transmission model ($\chi^2 \geq 5.84$ (1), $p = 0.02$). The τ_{AB}-free model more closely resembles a dominant major gene transmission pattern that describes a larger fraction of the PO and SS correlation and is therefore preferable. The model explained 38% of the variance.

Verbal fluency Verbal fluency showed moderate PO correlation (0.17 ± 0.10) and high SS correlation (0.34 ± 0.14; Figure 2C). This discrepancy might indicate the involvement of dominance or environmental effects in the siblings. Heritability was 0.53 (± 0.19). Commingling analysis indicated a mixture of three distributions for class-D and FPMM models (Table 3). The segregation analysis showed a significant rejection of the environmental ($\chi^2 = 23.09$ (3), $p < 0.01$), the Mendelian ($\chi^2 = 13.60$ (4), $p < 0.01$), and the homogeneous general model ($\chi^2 = 13.46$ (3), $p < 0.01$). The τ_{AB}-free model was not rejected by the reference model ($\chi^2 = 2.11$ (2), $p = 0.35$) and was most parsimonious (i.e. lowest AIC, Table 4; Table 3 in Supplement 3). In this τ_{AB}-free model, polygenic variance was close to zero, indicating little additional polygenic effects and τ_{AB} converged to 0.10 (± 0.07), indicating that the major effect may not be accounted for by Mendelian inheritance. The model explained about all of the variance of this trait in our families.

Table 5 Final segregation model parameters

Parameter	Parameter estimate ± SE			
Trait	Sensorimotor gating	Openness	Verbal fluency	Early visual perception
Transmission model	Homogeneous mendelian	τ_{AB}-free	τ_{AB}-free	τ_{AB}-free
Model settings	FPMM	Class D	FPMM	Class D
L	2	-	2	-
d	Two	Two	Three	Two
Mean AA	10.59 ± 0.14	9.28 ± 0.20	12.12 ± 0.20	10.12 ± 0.10
Mean AB	= Mean AA	= Mean AA	10.24 ± 0.20	= Mean AA
Mean BB	9.18 ± 0.36	10.61 ± 0.14	9.21 ± 0.22	8.35 ± 0.20
Variance	0.45 ± 0.09	0.54 ± 0.09	0.44 ± 0.10	0.62 ± 0.10
$\sigma_{poligenic}$	0[a]	-	0.02 ± 0.02	-
$\rho_{PO} = \rho_{SS}$	-	0.07 ± 0.08	-	0.21 ± 0.08
λ1	3.14 ± 1.14	1.89 ± 0.75	2.82 ± 0.86	-1[a]
q_A	0.37 ± 0.10	0.17 ± 0.06	0.47 ± 0.07	0.77 ± 0.07
τ_{AA}	[1]	[1]	[1]	[1]
τ_{AB}	[0.5]	0.72 ± 0.15	0.10 ± 0.07	0.28 ± 0.12
τ_{BB}	[0]	[0]	[0]	[0]

Note: parameters in square brackets are fixed. Means of genotypes AA, AB, BB are on a standardised scale, corrected for age and sex, with mean 10 and standard deviation of 1.

Abbreviations: d: number of distributions; FPMM: Finite Polygenic Mixed Models; L: number of loci fitted in FPMM; $\sigma_{polygenic}$: residual polygenic variance; $\rho_{PO} = \rho_{SS}$: residual familial correlations; λ1: transformation parameter; q_A: allele frequency; τ_{AA}, τ_{AB}, τ_{BB}: transmission probabilities for genotypes AA, AB, BB.

[a] Parameter converged to a bound.

Early visual perception Parent-offspring (0.22 ± 0.15) and SS correlation (0.23 ± 0.14) were nearly identical for early visual perception. Correspondingly, heritability was 0.37 (± 0.17; Figure 2D). Commingling analysis showed a mixture of two distributions for the class-D model, while the FPMM diverged to a one mean distribution (Table 3). In segregation analysis, none of the models was rejected by the reference model (Table 4; Table 4 in Supplement 3). The most parsimonious was the τ_{AB}-free model, which accounted for a larger fraction of the residual familial correlation, and could explain 16% of the variance.

Spatial working memory Both familial correlations (PO = 0.21 ± 0.12, SS = 0.22 ± 0.13) and heritability estimates (0.53 ± 0.19) (Figure 2E) were in the high range. Nevertheless, evidence for genetic transmission as tested in commingling analysis was lacking for both class-D as well as FPMM (Table 3). Therefore, we did not perform segregation analysis for spatial working memory.

Discussion

This family study in 180 subjects examined whether 13 candidate endophenotypes for schizophrenia are heritable and show a simpler mode of transmission, i.e., whether a major genetic effect can account for the heritability. Our results show that of the 13 candidate endophenotypes studied, only five show moderate familial correlations, i.e., sensorimotor gating, openness, verbal fluency, early visual perception, and spatial working memory, with equivalent heritability estimates (37%-54%). Only two of these five endophenotypes, sensorimotor gating and openness, reveal a simpler mode of inheritance, resembling a dominant pattern of transmission. Verbal fluency, early visual perception, and spatial working memory cannot be accounted for by a Mendelian pattern. Rather, polygenic, multifactorial, or environmental effects may underlie their variability.

Our heritability estimates for sensorimotor gating, openness, verbal fluency, and spatial working memory correspond to those previously reported,[20,52-55] while for the backward masking effect this is the first study to provide a heritability estimate.

Our finding of a dominant transmission pattern for sensorimotor gating is the first description of the mode of transmission for this trait in humans. Sensorimotor gating is measured psychophysiologically by prepulse inhibition, and refers to the attenuation of the magnitude of the startle response by a weak sensory (prepulse) stimulus. It enables healthy individuals to focus attention on salient aspects of the environment while screening trivial stimuli. Deficits in PPI are strongly associated with schizophrenia. Kraepelin[56] already described patients with schizophrenia as having problems in focusing attention. Subsequent studies have shown patients with schizophrenia to show an inability in filtering sensory stimuli, leading to overstimulation of higher brain regions.[57,58] This may ultimately result in the symptoms of schizophrenia.[59] Prepulse inhibition may be related to reduced dopaminergic and enhanced noradrenergic activity[60] and to cortico-striato-pallido-thalamic dysfunction across multiple disorders.[32] Recently, a functional missense mutation in the neuregulin 1 gene was found to affect PPI in schizophrenia patients and control subjects.[61]

Our study is also the first to describe the mode of inheritance for openness, a new potential endophenotype for schizophrenia. Openness to experience is one of the major dimensions of personality, the Big Five,[62] and is often characterised as cognitive flexibility or exploration. We included openness in our study, as persons with psychotic experiences in the general population score high on openness,[63] suggesting an association to the vulnerability to schizophrenia. Moreover, openness is highly heritable,[53] which was confirmed by our results. Interestingly, openness is the only personality trait linked to the functions of the dorsolateral prefrontal cortex,[64] a structure associated with schizophrenia and dopamine transmission.[65] Patients with schizophrenia

tend to score lower on openness,[33,34] with negative symptoms correlating negatively with openness, while positive symptoms show a positive correlation.[66] Indeed, descriptions of closeness (as opposed to openness as described by the NEO-FFI), such as: 'muted emotional responses,' fit with the clinical phenotype of schizophrenia patients having negative symptoms, whereas descriptions of openness, such as 'curious about both inner and outer world,' accords with positive symptoms, such as hallucinations or delusions. Openness needs to be characterised in relatives of schizophrenic patients to establish it as a candidate endophenotype for schizophrenia. Also, studying personality dimensions may be problematic among patients with psychosis; therefore, studying relatives rather than patients may avoid any potential disease-related response tendencies.

Our failure to find evidence for a genetic pattern of inheritance for spatial working memory conflicts with the findings of Tuulio-Henriksson et al.[20] that a low mean number of loci (1.03) underlies this trait. Moreover, spatial working memory has been linked to chromosome 1q.[67] The isolated population used in Tuulio-Henriksson's study may explain the larger contribution to a major gene effect. Furthermore, the two variables used (our sum of the forward and backward conditions versus their backward condition score) could have different genetic influences. Lastly, our result for spatial working memory may be a false negative, which occurs in a substantial proportion of commingling analyses (45% and 22%)[47] when used as a screening method as part of the segregation analysis.

We observed that the familial correlations for eight endophenotypes were lower than expected, in particular for P50, delayed verbal memory, set shifting, and sustained attention. Concerning P50, others have reported heritability estimates ranging from 30% to 76%,[14,68,69] whereas our results are similar to the nonsignificant heritability estimate of 0.10 of a large consortium study (Consortium on the Genetics of Schizophrenia [COGS]).[55] Presumably, the ratio score measured in these studies has low test-retest reliability,[68] which may explain the contradictory results. Using an alternative measure of P50, however (i.e., the difference between the test and conditioning amplitudes), as proposed by Anokhin et al.,[68] did not improve our familial correlations for P50. With regard to delayed verbal memory, heritability ranges from low to high (e.g., 7% to 66%),[12,13,20] and estimates have been lower than for other cognitive traits.[70,71] Regarding set shifting, heritability estimates are not available for Trailmaking part B-A (part B: 41% and 50%).[54,72] For sustained attention, only one study reported moderate (preliminary) heritability estimates on the Continuous Performance Test – Identical Pairs (CPT-IP).[73] Thus, for several endophenotypes, the few available heritability estimates have given conflicting results. Larger studies[55] are needed to further elucidate the underlying genetic variation for some of these tests.

There are some limitations to our study. Firstly, since we used multiply affected pedigrees, we cannot generalise our findings to the general population. Also, family size may have influenced parameter estimates in our study, and large families may therefore have been more informative. Thus, replication in systematically ascertained pedigrees or in the general population is needed. Secondly, the affected individuals in our analysis will constitute a healthier group than the schizophrenia patient population, simply by being able to participate. This bias is common and will apply to most family studies using cognitive traits. Thirdly, eight patients were diagnosed with schizoaffective disorder, which is not likely to influence our results since this phenotype shares genetic liability and risk factors with schizophrenia.[74] Additionally, our assessment of PPI, without the inclusion of several interstimulus intervals and a background noise level, may not have been optimal for schizophrenia research.[75] Although the startle effect will be robust to minor differences in settings and procedures,[76] our heritability results may thus be underestimated.

A limitation of segregation analysis is that genetic models including more complex mechanisms such as dynamic mutation, mitochondrial inheritance, or genomic imprinting are not considered and thus will not be detected.[77] Also, environmental effects can mimic Mendelian segregation patterns.[78] Even so, segregation analysis is a useful approach to direct further exploration of these families and to investigate the genetic characteristics of a trait prior to molecular genetic studies.

The strength of our study is the use of multigenerational, multiply affected pedigrees, which are potentially more informative in revealing major gene action. Moreover, by using multigenerational pedigrees rather than first-degree relatives only, our heritability estimates are less likely to be inflated by shared environmental effects.[13] Furthermore, the combination of three different analyses adds strength to our inheritance findings and thus allows a critical evaluation of several candidate endophenotypes for schizophrenia.

In summary, only 5 of 13 candidate endophenotypes for schizophrenia show the moderate familial correlations expected of candidate endophenotypes. However, of these, only two endophenotypes showed a simpler pattern of inheritance. Thus, one should be cautious about incorporating endophenotypes in genetic research, as they may not carry the advantage of a simpler mode of inheritance. Sensorimotor gating and openness appear to be promising candidate endophenotypes for genetic research in schizophrenia.

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Supplemental material cited in this article is available online.

Online Supplementary Material

Supplement 1

Methods

(In alphabetical order, if not given in the main document)

Backward Masking

The computerised visual backward masking battery developed by Michael Green et al (1) was administered on a standard PHILIPS PC running in Windows, with a 17″ Iiyama monitor driven at 150 Hz, 90 cm from the participant in a dimly-lit room. Dark gray target stimuli (0.29° visual angle) appeared on a white background at one of four corners of a square centered at the fixation (upper left, upper right, lower left, lower right) and consisted of a square with a gap on either the top, bottom or left side. A mask (1.14° visual angle from fixation) consisted of four sets of four smaller squares overlapping the four possible target locations. First the critical stimulus intensity (CSI) was determined for each participant as the grey scale value that yields performance of about 84% accuracy. Each task trial consisted of a 300 ms fixation cross at the center of visual display, 100 ms of blank screen, a 13.3 msec target stimulus, a varying stimulus onset asynchrony (SOA), and a mask. In the location condition participants indicated where the target appeared and in the identification condition which target appeared, with mask stimulus durations of respectively 26.7 and 6.7 ms. Responses were given vocally by the participant and entered on a keyboard by the experimenter. The quadrants for the location task were displayed on the rim of the monitor. Each condition consisted of 12 practice trials and 96 test trials consisting of 12 trials per SOA. The SOA (duration from the onset of the stimulus to the onset of the mask) varied

from -80 to 80 ms with increments of 13.3 ms, interspersed with 12 unmasked trials, and 24 additional trials with a SOA -120 or 120 ms in the identification condition. Only positive SOA's trials indicating backward masking were analysed. Performance on the interspersed unmasked trials was relatively high (96.1 and 83.3 for target location and low-energy-masking identification, respectively) and similar to Green et al.[1]

California Verbal Learning Test (CVLT)

The CVLT[2] is a test of verbal learning and memory. During assessment the participant listens to a woman's voice reading aloud (on tape) a list of 16 items five times and is asked on every occasion to repeat as many items as possible in random/free order. Immediate recall is the total number of words recalled on trials 1 through 5. Short delay recall is the number of words recalled after presentation and recollection of an interfering list. Delayed free recall is the number of words recalled after a 20-30 minute delay.

Continuous Performance Test – Identical Pairs (CPT-IP)

Continuous performance was assessed using the CPT-IP.[3] During assessment, the participant has to indicate as fast as possible, by lifting a finger from the mouse-button, whenever two consecutive stimuli look exactly alike. A trial consists of a 50 ms presentation of the stimulus (150 verbal stimuli: four-digit numbers in the verbal condition and 150 spatial stimuli: nonsense shapes in the spatial condition) followed by 950 ms of dark time. Trials are presented in a continuous sequence.

Digit span

The Digit Span subtest of the Wechsler Adult Intelligence Scale (WAIS)[4] is a test of verbal attention and verbal working memory. The test involves increasing sequences of digits pronounced by the experimenter at a one-per-second rate that are repeated by the participant in the same order (forwards) or in reverse order (backwards condition). The score equals the sum of correct repetitions in both conditions.

Facial Recognition Test (FRT)

Facial recognition was assessed using the FRT.[5] The target (a 'front-view' picture of a face) is depicted simultaneously with six test stimuli (six different faces including the target). Six trials are in an identical 'front-view', four at a different angle, and three at different angle and with different lighting conditions. The participant indicates a match verbally or by pointing, without time constraints. Scores are transformed to a long version score and corrected for age.[5]

Neuroticism

Neuroticism was measured as part of the Neuroticism-Extroversion-Openness - Five Factor Inventory (NEO-FFI)[6,7] a 60-item questionnaire that measures the major dimensions of personality, the Big Five.

P50 suppression

The auditory stimuli were gated almost instantaneously (rise/fall, 0.1 ms) and presented binaurally through stereo insert earphones (Eartone ABR). The software settings were calibrated by means of an artificial ear (Brűel and Kjær, type 4152) to make sure that the stimulus intensities at the subject's ear were the intended intensities. Each subject was seated upright in a dentist chair in a dimly lit sound-isolated cabin with an ambient room noise level of 34 dB. Before the actual experimental block started 2 click pairs were presented as an audiometric test. After instruction a block of 36 click pairs with an interstimulus interval of 500 ms, and an intertrial interval of 10 s was presented. The clicks consisted of a white noise burst of 1.5 ms, with an intensity of 86 dB. The subjects were instructed to keep their eyes closed and to count the click pairs.
Recordings were made by means of the Active Two system (Biosemi, Amsterdam), and the EEG was sampled at 2048 Hz and stored as a continuous signal. Two electrodes in the electrode cap, the CMS (=common mode sense) and DRL (=driven right leg) provided an active ground. An electrode placed on the left mastoid was used as reference for EEG measurement. Data were resampled offline at 500 Hz.
All EEG data were analysed using the software package Brain Vision Analyser (Biosemi, Amsterdam) and filtered offline with a high-pass filter of 1 Hz, a low-pass filter of 30 Hz, and a Notch filter of 50 Hz. In order to compute ERPs, epochs from 100 ms pre-stimulus until 400 ms post-stimulus were extracted from the continuous data, and the baseline was corrected using the 100 ms registration prior to stimulus-onset. Electrooculogram (EOG) artefacts were removed.[8] EEG artifacts were removed if they were larger than 100 or –100 μV, if there was an amplitude difference per sample point larger than 50 μV or if the difference between maximum and minimum amplitudes in a window of 200 ms was smaller than 3 μV. Six participants were excluded because of lack of identifiable P50 response. Segments were averaged to produce separate average evoked response potential (ERP) waveforms for the conditioning and test click stimuli.
The P50 waves were identified and scored as described by:[9] P50 peaks elicited by the first (conditioning) stimulus were identified as the greatest positivity in a window between 40 and 90 ms after stimulus presentation. If more than one peak was identified, the later one was selected. The amplitude was assessed as being the difference between this peak and the preceding trough, the latency was assessed as being the time from the onset of the conditioning stimulus to the maxi-

mum amplitude of this peak. The P50 peak elicited by the second (testing) stimulus was assessed accordingly, with a further constraint that its peak latency had to lay in a window formed by the latency of the conditioning stimulus ± 10 ms. The P50 ratio was calculated as the amplitude of the P50 potential elicited by the testing stimulus divided by the amplitude elicited by the conditioning stimulus (T/C). We used truncated ratio scores (values over 10 were set to 10, similar to[10]) to prevent outliers from disproportionately affecting the results. N100 was assessed as the largest negative deflection in a post-stimulus window of 80 and 150 ms for the conditioning stimulus. The test stimulus was scored in a window formed by the latency of the conditioning stimulus ± 30 ms, and was divided by the conditioning stimulus to calculate N100 ratio scores (T/C).

Prepulse Inhibition (PPI)

The prepulse and startle stimuli were bursts of white noise (duration 25 and 30 ms, intensity 87 dB and 107 dB, respectively), with a fixed interstimulus interval of 120 ms. The stimuli were gated almost instantaneously (rise/fall time, 0.1 ms) and presented binaurally through stereo insert earphones (Eartone ABR). The software settings were calibrated by means of an artificial ear (Brűel and Kjær, type 4152) to make sure that the stimulus intensities at the subject's ear were the intended intensities. Each subject was seated upright in a dentist chair in a dimly lit sound-isolated cabin with an ambient room noise level of 34 dB. Before the actual start of the PPI assessment, four startle stimuli of rising intensity were presented, two of which were preceded by a prepulse stimulus, to accustom the subjects to loud noises. The actual experiment consisted of a block of 24 randomized trials: 12 pulses (startle stimuli) preceded by a 'prepulse' stimulus and 12 pulses alone. The intertrial intervals were randomised between 12 and 23 s.

Recordings were made with the Active Two system (Biosemi, Amsterdam), and the electromyographic activity (EMG) was sampled at 2048 Hz and stored as a continuous signal. The EMG of the right orbicularis inferior muscle was recorded bipolarly; one electrode was located on the medial part of the muscle, the second one was located 0.5 cm exterior, in the direction of the outer canthus of the eye. Two electrodes in the electrode cap, the CMS (=common mode sense) and DRL (=driven right leg) provided an active ground in this system. Data were resampled offline at 500 Hz.

EMG data were analysed using the software package Brain Vision Analyser (Biosemi, Amsterdam) and filtered offline with a high-pass filter of 30 Hz and a low-pass-filter of 200 Hz. Epochs from -50 ms pre-stimulus until 200 ms post-stimulus were extracted from the continuous data, and the baseline was corrected using the data for 50 ms prior to stimulus-onset. Thereafter, the data were rectified. Last, assessment of the maximal peak amplitude and PPI quantification took

place within a window of 20–120 ms after stimulus onset, excluding the first startle and negative PPI's. We did not perform hearing tests nor employ a startle non-responder criterion. PPI was defined as the percent reduction in startle magnitude of prepulse-pulse trials compared to the pulse alone trials (PPI=100(1-pp/p), where pp indicates amplitude over prepulse-pulse trials and p indicates amplitude over pulse alone trials).

Purdue Pegboard Test

The Purdue pegboard test[11] is a test of timed motor speed and motor co-ordination sensitive to subtle psychomotor dysfunction. Scores equal the sum of the number of pegs placed in the holes of a board in three 30 seconds sessions, with each hand separately and finally bimanually. Arthritis was an exclusion criterion for the Purdue pegboard test.

Trail Making Test (TMT)

Set shifting was measured using the TMT, as the difference between part B and A.[12] In part A, the task requires the participant to draw a line connecting numbered circles successively as fast as possible without lifting the pencil. Part B involves connecting both lettered and numbered circles successively alternating between the two sequences. Completion time is used as the measure of performance.

Verbal Fluency (letter condition)

During the Letter Fluency task,[13] participants are asked to generate as many words beginning with the letter "n" and subsequently with "a" as possible within 60 seconds. The measure of performance score is the sum of the number of words.

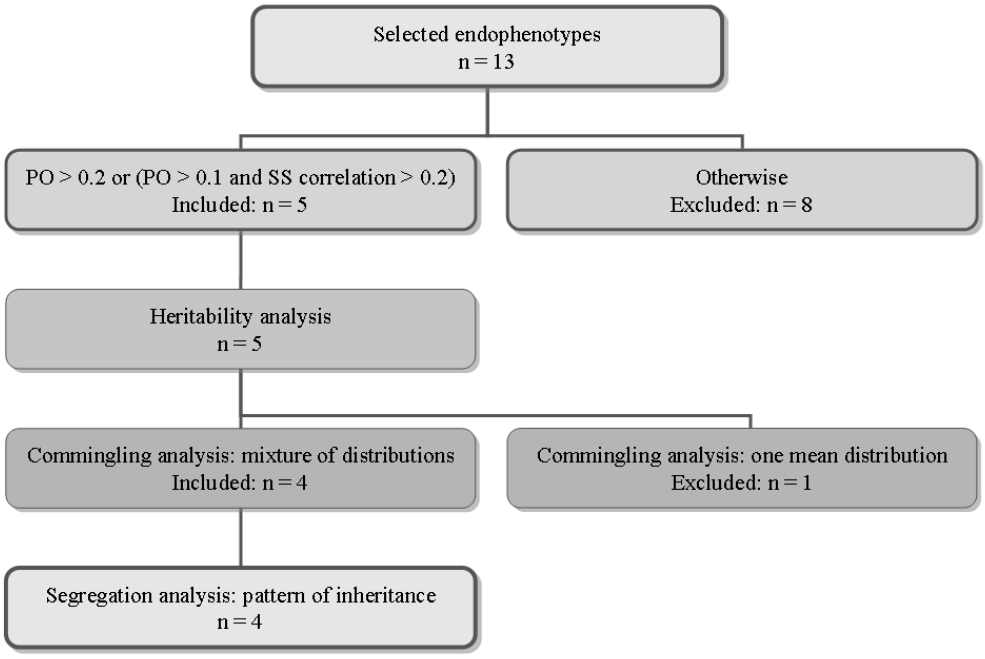
References

1. Green MF, Nuechterlein KH, Breitmeyer B (2002): Development of a computerized assessment for visual masking. *Int J Methods Psychiatr Res* 11: 83-89.
2. Mulder Jl, Dekker R, Dekker PH (1996): *California Verbal Learning Test (Dutch version).* Lisse, Swets & Zeitlinger.
3. Rosvold HE, Mirsky AF, Sarason I, Bransome ED Jr, Beck LH (1956): A continuous performance test of brain damage. *J Consult Psychol* 20: 343-350.
4. Stinissen J, Willems PJ, Coetsier P, Hulsman WL (1970): *Dutch adaption of the Wechsler Adult Intelligent Scale.* Amsterdam, Swets & Zeitlinger.
5. Benton AL, Sivan AB, Hamsher K, Varney NR, Spreen O (1994): *Contributions to neuropsychological assessment 2nd edition.* Oxford University Press, Inc.
6. Costa PT Jr., McCrae RR (1992): *Revised NEO Personality Inventory (NEO-PI-R) and NEO Five-Factor Inventory (NEO-FFI) professional manual*. Odessa, FL: Psychological Assessment Resources, Inc.
7. Hoekstra HA, Ormel J, de Fruyt F (1996): *NEO PI-R; NEO FFI; Big Five Persoonlijkheidsvragenlijsten; Handleiding*. Lisse, Swets & Zeitlinger
8. Gratton G, Coles MG, Donchin E (1983): A new method for off-line removal of ocular artifact. *Electroencephalogr Clin Neurophysiol* 55: 468-484.

9. Cadenhead KS, Light GA, Geyer MA, Braff DL (2000): Sensory gating deficits assessed by the P50 event-related potential in subjects with schizotypal personality disorder. *Am J Psychiatry* 157: 55-59.
10. Purdue Research Foundation (1948): *Examiners manual for the Purdue Pegboard.* Chicago, Science Research Associates.
11. Reitan RM (1992): *Trailmaking test. Manual for administration and scoring.* Tuscon, Reitan Neuropsychology Laboratory.
12. Benton AL, Hamsher K (1976): *Multilingual Aphasia Examination.* Iowa City, University of Iowa.

Supplement 2

Figure S1 Flowchart of study design: cognitive measures included and excluded



Abbreviations: PO: parent-offspring; SS: sib-sib; n: number of tests.

Supplement 3

Table S1 Parameter estimations (± standard errors) for all segregation models of sensorimotor gating (FPMM, two means, two loci)

Parameter	Hom no transmission	Hom Mendelian	Hom general	τ_{AB}-free	General
μ_{AA}_μ_{AB}	9.17 ± 0.26	10.59 ± 0.14	9.03 ± 0.27	10.60 ± 0.14	9.09 ± 0.27
μ_{BB}	10.66 ± 0.14	9.18 ± 0.32	10.58 ± 0.13	9.22 ± 0.31	10.61 ± 0.13
σ	0.37 ± 0.07	0.45 ± 0.09	0.39 ± 0.07	0.45 ± 0.09	0.38 ± 0.07
$\sigma_{poligenic}$	0[a]	0[a]	0[a]	0[a]	0[a]
$\lambda 1$	3.33 ± 1.17	3.14 ± 1.14	2.75 ± 1.09	3.26 ± 1.14	2.97 ± 1.13
q_A	0.23 ± 0.07	0.37 ± 0.10	0.20 ± 0.06	0.39 ± 0.11	0.12 ± 0.07
τ_{AA}	-	[1]	0.96 ± 0.49	[1]	1[a]
τ_{AB}	-	[0.5]	0.33 ± 0.12[b]	0.43 ± 0.11	0.47 ± 0.17
τ_{BB}	-	[0]	0.08 ± 0.06	[0]	0.13 ± 0.07
-2lnL	350.22	348.87	346.14	348.51**	343.65
AIC	360.22	358.87	360.14	360.51**	357.65
N	5	5	7	6	7
χ^2 (df)	6.57 (2)	5.22 (2)	2.49 (0)	4.85 (1)	Ref.
p	0.04	0.07	nc	0.03	-

Abbreviations: hom: homologous; μ_{AA}, μ_{AB}, μ_{BB}: means of genotypes AA, AB, BB on a standardised scale, corrected for age and sex, with mean 10 and standard deviation of 1; σ: variance; $\sigma_{polygenic}$: residual polygenic variance; $\lambda 1$: transformation parameter; q_A: allele frequency; τ_{AA}, τ_{AB}, τ_{BB}: transmission probabilities for genotypes AA, AB, BB; -2lnL: -2log likelihood; AIC= An Information Criterion; N: number of estimated parameters; χ^2 (df) and p: chi square statistic, degrees of freedom and p-value compared to the general reference model; Ref: reference model; nc: not comparable.

Note: parameters in squared brackets were fixed.

[a] Parameter converged to a bound.

[b] Parameter depends on other parameter(s).

** Maximisation procedure did not complete cleanly; results may not be totally maximised.

Table S2 Parameter estimations (± standard errors) for all segregation models of openness (class-D, two mean)

Parameter	Hom no transmission	Hom Mendelian	Hom general	τ_{AB}-free	General
μ_{AA_AB}	9.34 ± 0.23	10.80 ± 0.16	9.52 ± 0.18	9.28 ± 0.20	9.51 ± 0.18
μ_{BB}	10.51 ± 0.17	9.45 ± 0.16	10.56 ± 0.14	10.61 ± 0.14	10.58 ± 0.16
σ	0.69 ± 0.12	0.50 ± 0.08	0.77 ± 0.12	0.54 ± 0.09	0.74 ± 0.15
$\rho_{PO} = \rho_{SS}$	0.56 ± 0.01	0.02 ± 0.09	0.54 ± 0.01	0.07 ± 0.08	0.55 ± 0.02
$\lambda 1$	2.01 ± 0.77	2.52 ± 0.81	2.18 ± 0.65	1.89 ± 0.75	2.24 ± 0.69
q_A	0.24 ± 0.08	0.28 ± 0.08	0.26 ± 0.08	0.17 ± 0.06	0.26 ± 0.08
τ_{AA}	-	[1]	1[a]	[1]	1[a]
τ_{AB}	-	[0.5]	0[a, b]	0.72 ± 0.15	0.03 ± 0.11
τ_{BB}	-	[0]	0.47 ± 0.17	[0]	0.45 ± 0.17
-2lnL	435.64	432.12	429.75	429.80	429.67
AIC	447.64	444.12	443.75	443.80	445.67
N	6	6	7	7	8
χ^2 (df)	5.96 (2)	2.45 (2)	0.08 (1)	0.13 (1)	Ref
p	0.05	0.29	0.78	0.72	-

For legend see table S1.

Table S3 Parameter estimations (± standard errors) for all segregation models of verbal fluency (FPMM, three means, two loci)

Parameter	Hom no transmission	Hom Mendelian	Hom general	τ_{AB}-free	General
μ_{AA}	12.86 ± 3.23	12.54 ± 0.50	12.36 ± 0.45	12.12 ± 0.20	12.11 ± 0.17
μ_{AB}	12.15 ± 0.34	10.65 ± 0.18	10.59 ± 0.17	10.24 ± 0.20	10.24 ± 0.15
μ_{BB}	9.76 ± 0.22	9.59 ± 0.15	9.52 ± 0.20	9.21 ± 0.22	9.10 ± 0.22
σ	0.69 ± 0.13	0.54 ± 0.10	0.49 ± 0.13	0.44 ± 0.10	0.36 ± 0.09
$\sigma_{\ poligenic}$	0.01 ± 0.02	0[a]	0[a]	0.02 ± 0.02	0.02 ± 0.02
$\lambda 1$	2.92 ± 0.95	2.44 ± 0.72	2.48 ± 0.72	2.82 ± 0.86	2.97 ± 0.65
q_A	0.02 ± 0.01	0.20 ± 0.07	0.22 ± 0.07	0.47 ± 0.07	0.48 ± 0.06
τ_{AA}	-	[1]	1[a]	[1]	0.95 ± 0.06
τ_{AB}	-	[0.5]	0.46 ± 0.10[b]	0.10 ± 0.07	0.09 ± 0.07
τ_{BB}	-	[0]	0.02 ± 0.06	[0]	0.05 ± 0.06
-2lnL	378.55	369.06	368.93	357.58	355.47
AIC	392.55	381.06	382.93	373.58	375.47
N	7	6	7	8	10
χ^2 (df)	23.09 (3)	13.60 (4)	13.46 (3)	2.11 (2)	Ref.
p	<0.01	0.01	<0.01	0.35	-

For legend see table S1.

Table S4 Parameter estimations (± standard errors) for all segregation models of early visual perception (class-D, two means)

Parameter	Hom no transmission	Hom Mende-lian	Hom general	τ_{AB}-free	General
μ_{AA_AB}	11.56 ± 0.18	10.13 ± 0.10	11.56 ± 0.17	10.12 ± 0.10	11.56 ± 0.17
μ_{BB}	9.86 ± 0.11	8.36 ± 0.21	9.86 ± 0.11	8.35 ± 0.20	9.86 ± 0.11
σ	0.57 ± 0.09	0.62 ± 0.10	0.55 ± 0.09	0.62 ± 0.10	0.55 ± 0.09
$\rho_{PO}= \rho_{SS}$	0.38 ± 0.10	0.20 ± 0.09	0.36 ± 0.09	0.21 ± 0.08	0.36 ± 0.09
$\lambda 1$	3.73 ± 1.10	-1[a]	3.86 ± 0.98	-1[a]	3.86 ± 0.98
q_A	0.07 ± 0.02	0.69 ± 0.07	0.03 ± 0.03	0.77 ± 0.07	0.03 ± 0.03
τ_{AA}	-	[1]	0.04 ± 0.71	[1]	0.04 ± 0.71
τ_{AB}	-	[0.5]	0[a, b]	0.28 ± 0.12	0[a]
τ_{BB}	-	[0]	0.09 ± 0.03	[0]	0.09 ± 0.03
-2lnL	336.3	337.5	334.6	335.1	334.6
AIC	348.3	347.5	350.6	347.1	350.6
N	6	5	8	6	8
χ^2 [d]	1.77 (2)	2.93 (3)	0.00 (0)	0.50 (2)	Ref.
p	0.41	0.40	nc	0.78	-

For legend see table S1.

References

1. Cardno AG, Gottesman II (2000): Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. *Am J Med Genet* 97: 12-17.
2. Norton N, Williams HJ, Owen MJ (2006): An update on the genetics of schizophrenia. *Curr Opin Psychiatry* 19: 158-164.
3. Fanous AH, Kendler KS (2005): Genetic heterogeneity, modifier genes, and quantitative phenotypes in psychiatric illness: searching for a framework. *Mol Psychiatry* 10: 6-13.
4. Freedman R, Leonard S, Olincy A, Kaufmann CA, Malaspina D, Cloninger CR et al (2001): Evidence for the multigenic inheritance of schizophrenia. *Am J Med Genet* 105: 794-800.
5. Gottesman II, Gould TD (2003): The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 160: 636-645.
6. Weeks DE, Lathrop GM (1995): Polygenic disease: methods for mapping complex disease traits. *Trends Genet* 11: 513-519.
7. Freedman R, Leonard S, Gault JM, Hopkins J, Cloninger CR, Kaufmann CA et al (2001): Linkage disequilibrium for schizophrenia at the chromosome 15q13-14 locus of the alpha7-nicotinic acetylcholine receptor subunit gene (CHRNA7). *Am J Med Genet* 105: 20-22.
8. Paunio T, Tuulio-Henriksson A, Hiekkalinna T, Perola M, Varilo T, Partonen T et al (2004): Search for cognitive trait components of schizophrenia reveals a locus for verbal learning and memory on 4q and for visual working memory on 2q. *Hum Mol Genet* 13: 1693-1702.
9. Williams JT, Begleiter H, Porjesz B, Edenberg HJ, Foroud T, Reich T et al (1999): Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. II. Alcoholism and event-related potentials. *Am J Hum Genet* 65: 1148-1160.
10. Boos HB, Aleman A, Cahn W, Hulshoff Pol HE, Kahn RS (2007): Brain volumes in relatives of patients with schizophrenia: a meta-analysis. *Arch Gen Psychiatry* 64: 297-304.
11. Sitskoorn MM, Aleman A, Ebisch SJ, Appels MC, Kahn RS (2004): Cognitive deficits in relatives of patients with schizophrenia: a meta-analysis. *Schizophr Res* 71: 285-295.
12. Cannon TD, Huttunen MO, Lonnqvist J, Tuulio-Henriksson A, Pirkola T, Glahn D et al (2000): The inheritance of neuropsychological dysfunction in twins discordant for schizophrenia. *Am J Hum Genet* 67: 369-382.
13. Gur RE, Nimgaonkar VL, Almasy L, Calkins ME, Ragland JD, Pogue-Geile MF et al (2007): Neurocognitive endophenotypes in a multiplex multigenerational family study of schizophrenia. *Am J Psychiatry* 164: 813-819.
14. Hall MH, Schulze K, Rijsdijk F, Picchioni M, Ettinger U, Bramon E et al (2006): Heritability and reliability of P300, P50 and duration mismatch negativity. *Behav Genet* 36: 845-857.
15. Kuntsi J, Rogers H, Swinard G, Borger N, van der Meere J, Rijsdijk F et al (2006): Reaction time, inhibition, working memory and 'delay aversion' performance: genetic influences and their interpretation. *Psychol Med* 36: 1613-1624.
16. Grove WM, Clementz BA, Iacono WG, Katsanis J (1992): Smooth pursuit ocular motor dysfunction in schizophrenia: evidence for a major gene. *Am J Psychiatry* 149: 1362-1368.
17. Matthysse S, Holzman PS, Lange K (1986): The genetic transmission of schizophrenia: application of Mendelian latent structure analysis to eye tracking dysfunctions in schizophrenia and affective disorder. *J Psychiatr Res* 20: 57-67.
18. Waldo MC, Carey G, Myles-Worsley M, Cawthra E, Adler LE, Nagamoto HT et al (1991): Codistribution of a sensory gating deficit and schizophrenia in multi-affected families. *Psychiatry Res* 39: 257-268.
19. Sham PC, Morton NE, Muir WJ, Walker M, Collins A, Shields DC et al (1994): Segregation analysis of complex phenotypes: an application to schizophrenia and auditory P300 latency. *Psychiatr Genet* 4: 29-38.
20. Tuulio-Henriksson A, Haukka J, Partonen T, Varilo T, Paunio T, Ekelund J et al (2002): Heritability and number of quantitative trait loci of neurocognitive functions in families with schizophrenia. *Am J Med Genet* 114: 483-490.
21. Arolt V, Lencer R, Nolte A, Muller-Myhsok B, Purmann S, Schurmann M et al (1996): Eye tracking dysfunction is a putative phenotypic susceptibility marker of schizophrenia and maps to a locus on chromosome 6p in families with multiple occurrence of the disease. *Am J Med Genet* 67: 564-579.

22. Arolt V, Lencer R, Purmann S, Schurmann M, Muller-Myhsok B, Krecker K et al (1999): Testing for linkage of eye tracking dysfunction and schizophrenia to markers on chromosomes 6, 8, 9, 20, and 22 in families multiply affected with schizophrenia. *Am J Med Genet* 88: 603-606.
23. Matthysse S, Holzman PS, Gusella JF, Levy DL, Harte CB, Jorgensen A et al (2004): Linkage of eye movement dysfunction to chromosome 6p in schizophrenia: additional evidence. *Am J Med Genet B Neuropsychiatr Genet* 128: 30-36.
24. Coon H, Plaetke R, Holik J, Hoff M, Myles-Worsley M, Waldo M et al (1993): Use of a neurophysiological trait in linkage analysis of schizophrenia. *Biol Psychiatry* 34: 277-289.
25. Freedman R, Coon H, Myles-Worsley M, Orr-Urtreger A, Olincy A, Davis A et al (1997): Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proc Natl Acad Sci U S A* 94: 587-592.
26. Myles-Worsley M, Coon H, McDowell J, Brenner C, Hoff M, Lind B et al (1999): Linkage of a composite inhibitory phenotype to a chromosome 22q locus in eight Utah families. *Am J Med Genet* 88: 544-550.
27. Baron M (2001): Genetics of schizophrenia and the new millennium: progress and pitfalls. *Am J Hum Genet* 68: 299-312.
28. National Institute of Mental Health (1991): *Molecular Genetics Initiative: Family Interview for Genetic Studies.* Bethesda, MD, National Institute of Mental Health.
29. Andreasen NC, Flaum M, Arndt S (1992): The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnosis and psychopathology. *Arch Gen Psychiatry* 49: 615-623.
30. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E et al (1998): The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 59 Suppl 20: 22-33.
31. Tsuang MT, Stone WS, Faraone SV (1999): The genetics of schizophrenia. *Curr Psychiatry Rep* 1: 20-24.
32. Braff DL, Geyer MA, Swerdlow NR (2001): Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)* 156: 234-258.
33. Bagby RM, Bindseil KD, Schuller DR, Rector NA, Young LT, Cooke RG et al (1997): Relationship between the five-factor model of personality and unipolar, bipolar and schizophrenic patients. *Psychiatry Res* 70: 83-94.
34. Camisa KM, Bockbrader MA, Lysaker P, Rae LL, Brenner CA, O'Donnell BF (2005): Personality traits in schizophrenia and related personality disorders. *Psychiatry Res* 133: 23-33.
35. Costa PT Jr., McCrae RR (1992): *Revised NEO Personality Inventory (NEO-PI-R) and NEO Five-Factor Inventory (NEO-FFI) professional manual.* Odessa, FL: Psychological Assessment Resources, Inc.
36. Hoekstra HA, Ormel J, de Fruyt F (1996): *NEO PI-R; NEO FFI; Big Five Persoonlijkheidsvragenlijsten; Handleiding.* Lisse: Zwets & Zeitlinger B.V.
37. Luteijn F, Van der Ploeg F (1983): *The Groninger Intelligence Test (in Dutch).* Lisse: Swets & Zeitlinger.
38. Bokat CE, Goldberg TE (2003): Letter and category fluency in schizophrenic patients: a meta-analysis. *Schizophr Res* 64: 73-78.
39. Henry JD, Crawford JR (2005): A meta-analytic review of verbal fluency deficits in schizophrenia relative to other neurocognitive deficits. *Cognit Neuropsychiatry* 10: 1-33.
40. Green MF, Nuechterlein KH, Breitmeyer B, Tsuang J, Mintz J (2003): Forward and backward visual masking in schizophrenia: influence of age. *Psychol Med* 33: 887-895.
41. Green MF, Nuechterlein KH, Breitmeyer B (2002): Development of a computerized assessment for visual masking. *Int J Methods Psychiatr Res* 11: 83-89.
42. Wechsler D (1997): *Wechsler Memory Scale,* 3rd ed. San Antonio, TX, the Psychological Corporation.
43. Lee J, Park S (2005): Working memory impairments in schizophrenia: a meta-analysis. *J Abnorm Psychol* 114: 599-611.
44. S.A.G.E. Statistical Analysis for Genetic Epidemiology, Release 5.3. 2006. http://genepi.cwru.edu/.
45. Almasy L, Blangero J (1998): Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 62: 1198-1211.

46. Box GEP, Cox DR (1964): An analysis of transformations (with discussion). *Journal of the Royal Statistical Society, Series B, Methodological* 26: 211.
47. Kwon JM, Boehnke M, Burns TL, Moll PP (1990): Commingling and segregation analyses: comparison of results from a simulation study of a quantitative trait. *Genet Epidemiol* 7: 57-68.
48. Bonney GE (1984): On the statistical determination of major gene mechanisms in continuous human traits: regressive models. *Am J Med Genet* 18: 731-749.
49. Fernando RL, Stricker C, Elston RC (1994): The Finite Polygenic Mixed-Model - An Alternative Formulation for the Mixed-Model of Inheritance. *Theoretical and Applied Genetics* 88: 573-580.
50. Akaike H (1974): A new look at the statistical model identification. *IEEE Transactions on Automatic Control AC* 19: 716-723.
51. Stein CM, Nshuti L, Chiunda AB, Boom WH, Elston RC, Mugerwa RD et al (2005): Evidence for a Major Gene Influence on Tumor Necrosis Factor-alpha Expression in Tuberculosis: Path and Segregation Analysis. *Hum Hered* 60: 109-118.
52. Anokhin AP, Heath AC, Myers E, Ralano A, Wood S (2003): Genetic influences on prepulse inhibition of startle reflex in humans. *Neurosci Lett* 353: 45-48.
53. Bouchard TJ, Jr., McGue M (2003): Genetic and environmental influences on human psychological differences. *J Neurobiol* 54: 4-45.
54. Swan GE, Carmelli D (2002): Evidence for genetic mediation of executive control: a study of aging male twins. *J Gerontol B Psychol Sci Soc Sci* 57: 133-143.
55. Greenwood TA, Braff DL, Light GA, Cadenhead KS, Calkins ME, Dobie DJ et al (2007): Initial heritability analyses of endophenotypic measures for schizophrenia: the consortium on the genetics of schizophrenia. *Arch Gen Psychiatry* 64: 1242-1250.
56. Kraepelin E (1913): *Psychiatrie, ein lehrbuch für studierende und ärzte* , 8th ed. Leibzig: J.A. Barth.
57. Venables PH (1964): Input dysfunction in schizophrenia. In Maher BA, editor. *Progress in Experimental Personality Research*. New York, London: Academic Press, pp 1-47.
58. McGhie A, Chapman J (1961): Disorders of attention and perception in early schizophrenia. *Br J Med Psychol* 34: 103-116.
59. Braff DL, Geyer MA (1990): Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch Gen Psychiatry* 47: 181-188.
60. Oranje B, Kahn RS, Kemner C, Verbaten MN (2004): Modulating sensorimotor gating in healthy volunteers: the effects of desipramine and haloperidol. *Psychiatry Res* 127: 195-205.
61. Hong LE, Wonodi I, Stine OC, Mitchell BD, Thaker GK (2008): Evidence of Missense Mutations on the Neuregulin 1 Gene Affecting Function of Prepulse Inhibition. *Biol Psychiatry* 63: 17-23.
62. Digman JM (1990): Personality Structure - Emergence of the 5-Factor Model. *Annual Review of Psychology* 41: 417-440.
63. Laroi F, Van der Linden M, De Fruyt F, van Os J, Aleman A (2006): Associations between delusion proneness and personality structure in non-clinical participants: comparison between young and elderly samples. *Psychopathology* 39: 218-226.
64. DeYoung CG, Peterson JB, Higgins DM (2005): Sources of openness/intellect: cognitive and neuropsychological correlates of the fifth factor of personality. *J Pers* 73: 825-858.
65. Weinberger DR, Egan MF, Bertolino A, Callicott JH, Mattay VS, Lipska BK et al (2001): Prefrontal neurons and the genetics of schizophrenia. *Biol Psychiatry* 50: 825-844.
66. Ross SR, Lutz CJ, Bailley SE (2002): Positive and negative symptoms of schizotypy and the Five-factor model: a domain and facet level analysis. *J Pers Assess* 79: 53-72.
67. Gasperoni TL, Ekelund J, Huttunen M, Palmer CG, Tuulio-Henriksson A, Lonnqvist J et al (2003): Genetic linkage and association between chromosome 1q and working memory function in schizophrenia. *Am J Med Genet* 116B: 8-16.
68. Anokhin AP, Vedeniapin AB, Heath AC, Korzyukov O, Boutros NN (2007): Genetic and environmental influences on sensory gating of mid-latency auditory evoked responses: a twin study. *Schizophr Res* 89: 312-319.
69. Young DA, Waldo M, Rutledge JH, III, Freedman R (1996): Heritability of inhibitory gating of the P50 auditory-evoked potential in monozygotic and dizygotic twins. *Neuropsychobiology* 33: 113-117.
70. Antila M, Tuulio-Henriksson A, Kieseppa T, Soronen P, Palo OM, Paunio T et al (2007): Heritability of cognitive functions in families with bipolar disorder. *Am J Med Genet B Neuropsychiatr Genet* 144: 802-808.

71. Sleegers K, de Koning I, Aulchenko YS, van Rijn MJ, Houben MP, Croes EA et al (2007): Cerebrovascular risk factors do not contribute to genetic variance of cognitive function: the ERF study. *Neurobiol Aging* 28: 735-741.
72. Waldman ID (2005): Statistical approaches to complex phenotypes: evaluating neuropsychological endophenotypes for attention-deficit/hyperactivity disorder. *Biol Psychiatry* 57: 1347-1356.
73. Cornblatt BA, Risch NJ, Faris G, Friedman D, Erlenmeyer-Kimling L (1988): The Continuous Performance Test, identical pairs version (CPT-IP): I. New findings about sustained attention in normal families. *Psychiatry Res* 26: 223-238.
74. Owen MJ, Craddock N, Jablensky A (2007): The genetic deconstruction of psychosis. *Schizophr Bull* 33: 905-911.
75. Hsieh MH, Swerdlow NR, Braff DL (2006): Effects of background and prepulse characteristics on prepulse inhibition and facilitation: implications for neuropsychiatric research. *Biol Psychiatry* 59: 555-559.
76. Swerdlow NR, Sprock J, Light GA, Cadenhead K, Calkins ME, Dobie DJ et al (2007): Multi-site studies of acoustic startle and prepulse inhibition in humans: Initial experience and methodological considerations based on studies by the Consortium on the Genetics of Schizophrenia. *Schizophr Res* 92: 237-251.
77. Craddock N, Van Eerdewegh P, Reich T (1997): Single major locus models for bipolar disorder are implausible. *Am J Med Genet* 74: 18-20.
78. McGuffin P, Huckle P (1990): Simulation of Mendelism revisited: the recessive gene for attending medical school. *Am J Hum Genet* 46: 994-999.

Chapter 3

Genetic Overlap Among Intelligence and Other Candidate Endophenotypes for Schizophrenia

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Abstract

Background A strategy to improve genetic studies of schizophrenia involves the use of endophenotypes. Information on overlapping genetic contributions among endophenotypes may provide additional power, reveal biological pathways and have practical implications for genetic research. Several cognitive endophenotypes, including intelligence, are likely to be modulated by overlapping genetic influences.

Methods We quantified potential genetic and environmental correlations among endophenotypes for schizophrenia, including sensorimotor gating, openness, verbal fluency, early visual perception, spatial working memory, and intelligence, using variance component models in 35 patients and 145 relatives from 25 multigenerational Dutch families multiply affected with schizophrenia.

Results Significant correlations were found between spatial working memory and intelligence (0.45), verbal fluency and intelligence (0.36), verbal fluency and spatial working memory (0.20), and early visual perception and spatial working memory (0.19). A strong genetic correlation (0.75) accounted for 76% of the variance shared between spatial working memory and intelligence. Significant environmental correlations were found between verbal fluency and openness (0.50), and between verbal fluency and spatial working memory (0.58). Sensorimotor gating and openness showed few genetic or environmental correlations with other endophenotypes.

Conclusions Our results suggest that intelligence strongly overlaps genetically with a known cognitive endophenotype for schizophrenia. Intelligence may thus be a promising endophenotype for genetic research in schizophrenia, even though the underlying genetic mechanism may still be complex. In contrast, sensorimotor gating and openness appear to represent separate genetic entities with simpler inheritance patterns and may therefore augment the detection separate genetic pathways contributing to schizophrenia.

Introduction

Endophenotypes have been advocated for improving genetic research on complex disorders such as schizophrenia.[1,2] Characteristics of endophenotypes that help to increase power in genetic research include their simpler phenotype, putative simpler mode of transmission, quantitative nature, and potential to identify the unaffected but potentially gene-carrying relatives.[3,4] In addition, these characteristics may aid in revealing underlying biological pathways. A wide range of traits have been proposed as endophenotypes for schizophrenia, including abnormalities in cognition, perception, and psychophysiology.[5-7] Most of these traits have been shown to fulfil the criteria for endophenotypes as defined by Gottesman and Gould,[4] such as reliability, stability, and heritability.[8,9] Some endophenotypes, such as P50, a psychophysiological sensory motor task, and verbal learning and memory, have already been successfully used in gene-finding studies.[10,11]

Endophenotypes for schizophrenia may be (partially) influenced by the same genes, for instance, because they measure part of the same construct or because the underlying genes have a broad impact on the brain, which, in turn, affects multiple functions. Information on genetic overlap among various endophenotypes could reveal underlying biological pathways and has practical implications and advantages for genetic research. First, if one gene contributes to multiple endophenotypes (i.e., pleiotropy) then a combination of these endophenotypes may be used to detect this gene.[12-14] Second, traits that show less genetic overlap with others may be more informative in finding (separate) genetic variants. Consequently, it is worthwhile to investigate which endophenotypes share genetic factors, i.e., correlate genetically, and which do not.

Few studies have explored the extent to which overlapping genetic or environmental factors contribute to candidate endophenotypes for schizophrenia.[15-19] Greenwood et al.[15] investigated a range of neurocognitive measures in families affected with schizophrenia. Their results suggest that most neurocognitive measures are genetically correlated, while sensorimotor gating (a psychophysiological endophenotype) depends on separate genetic and environmental factors. Similarly, Hall et al.,[16] in healthy twins, showed that several psychophysiological endophenotypes, such as P50, P300, and mismatch negativity, constitute separate genetic entities. Furthermore, it has been found in several twin studies that intelligence is genetically correlated to working memory,[17,18] and brain volumes,[19] though not to P300.[17] Together these studies suggest that several cognitive endophenotypes for schizophrenia may overlap genetically, while others (such as psychophysiological traits) may be influenced by distinct genes. However, since most of these studies were conducted in the healthy population, it is unclear whether the same pattern of genetic overlap is present in families affected with schizophrenia. Moreover, the nature of the pat-

tern of genetic overlap among a different set of endophenotypes, such as intelligence and cognitive tasks, has not been studied.

Intelligence (or IQ) is a particularly interesting endophenotype when the potential genetic overlap among endophenotypes for schizophrenia is the focus of study. First, low (premorbid) IQ is associated with (genetic) risk for developing schizophrenia.[20-22] Furthermore, the heritability of intelligence is estimated to be high (60-80%),[23] though multiple genes are likely to be involved.[24] Finally, intelligence represents, by definition, the covariation among diverse measures of cognitive ability[25] and correlates with more elementary cognitive tasks.[26,27] Twin and adoption studies suggest that the correlation between intelligence and several cognitive tasks is largely genetic.[28-31] It is thus conceivable that intelligence will share genetic factors with other (cognitive) endophenotypes for schizophrenia. Indeed, as mentioned above, intelligence has been shown to be genetically related to spatial working memory[17,18] and brain volumes,[19] both candidate endophenotypes for schizophrenia.

The aim of this study is to investigate the extent of genetic overlap between six promising endophenotypes, including intelligence, in families affected with schizophrenia. Initially, 13 endophenotypes were selected for fulfilling criteria for endophenotypes,[4,32] such as heritability, stability and reliability. As reported earlier by us, 5 of these 13 endophenotypes showed moderate to high heritability (37% to 54%)[33] and were therefore selected for the current study: sensorimotor gating, openness, verbal fluency, early visual perception, and spatial working memory. For these five heritable endophenotypes as well as for intelligence, we calculated Pearson's correlations and quantified the shared genetic and environmental contributions to the variability of the traits in extended families multiply affected with schizophrenia.

Methods and materials

Participants

The participants and assessment have been described earlier.[33] In short, a total of 35 patients and 145 relatives from 25 multiplex multigenerational pedigrees of Dutch origin were recruited from the general population. Each pedigree comprised at least two members with a schizophrenia or schizoaffective disorder diagnosis of which at least one member's diagnosis was confirmed (see diagnostic assessment). Family size ranged from 2 to 21 relatives with a mean of 7.24 (sd = 4.93). The sample contained 653 pairings of relatives for whom genetic and diagnostic status was available, which includes all possible pairings within each family: 139 parent-offspring pairs, 165 sib pairs, 228 second-degree relatives and 121 third-degree relatives. No loops or consanguineous mating pairs were present. The mean age was 47.06 (sd = 16.75) and the proportion

of females was 55%. Nine patients and five relatives could not participate in endophenotypic measurement. Additionally, one patient and seven relatives fulfilled exclusion criteria. Exclusion criteria were: severe medical or neurological illness; history of closed-head injury; loss of consciousness longer than 30 minutes; history of alcohol abuse within last 6 months; diseases of the central nervous system and history of cerebrovascular accidents, dementia or delirium; age < 16; or IQ < 70. Four relatives could not identify the darkest grey scale of stimuli, which was an exclusion criterion for backward masking. Written informed consent was obtained from all participants after complete description of the study. The study was approved by the Medical Ethics Committee of the University Medical Center (UMC) Utrecht.

Diagnostic assessment

Each family was screened for the presence of diagnoses of schizophrenia or related disorders using the Family Interview for Genetic Studies (FIGS)[34] conducted by telephone with a family member. Patients were diagnosed by DSM-IV criteria on the basis of the Comprehensive Assessment of Symptoms and History (CASH), a semistructured diagnostic interview,[35] and by retrieving medical records. Psychiatric illness in relatives was assessed by means of the Mini-International Neuropsychiatric Interview (MINI-PLUS), a structured clinical interview of DSM axis 1 diagnoses.[36]

Endophenotypic assessment

The candidate endophenotypes were part of an extensive neuropsychological and psychophysiological test battery, which was conducted in the same order for every participant. Personality questionnaires were completed beforehand. The test battery was chosen on the basis of fulfilling as many criteria as possible for candidate endophenotypes, such as heritability, stability, and reliability.[4,32] In a previous study,[33] we showed 5 of the 13 endophenotypes to fulfil our selection criterion of moderate familial correlation (a parent-offspring correlation above 0.2 or higher than 0.1 when the sib-sib correlation was above 0.2), which was chosen to capture the most likely heritable traits for further analysis, i.e., sensorimotor gating, openness, verbal fluency, early visual perception, and spatial working memory. These traits showed equivalent heritability estimates (37%-54%). Additionally, we investigated the mode of inheritance for the five selected endophenotypes in a segregation analysis by estimating maximum likelihoods of transmission probabilities and allele frequencies for various inheritance models. We found a simpler Mendelian mode of inheritance for sensorimotor gating and openness, and more complex inheritance for the other three traits. These five promising endophenotypes were included in the present study and are described below.

Sensorimotor gating Prepulse inhibition (PPI) of the startle response is a measure of sensorimotor gating (SG), with a high inhibition score indicating better gating. Schizophrenia patients show less gating, i.e., lower inhibition scores, than control subjects.[37] In our sample, heritability (h^2) was 0.46 (standard error (SE) = 0.23).[33] Experimental task setup, signal recording, and assessment of PPI have been previously described.[33] Shortly, a startle is elicited by a 107 dB burst of white noise of 30 milliseconds (msec). A prepulse stimulus of 87 dB white noise for 25 msec precedes the startle stimulus in 50% of the trials to induce PPI. Prepulse inhibition is defined as the percent reduction in startle magnitude in the presence of the prepulse compared with the magnitude in the absence of the prepulse.

Openness to experience Openness to experience (OP) (openness) is often characterised as cognitive flexibility or exploration. Patients with schizophrenia score lower than control subjects on openness.[38,39] Openness was measured as part of the Neuroticism Extroversion Openness - Five Factor Inventory (NEO-FFI),[40] Dutch version,[41] a 60-item questionnaire that measures the major dimensions of personality, the Big Five. In our sample h^2 was 0.54 (SE = 0.13).[33]

Verbal fluency Category Fluency[42] is regarded as an executive task that gives an indication of verbal fluency (VF). Meta-analyses steadily show lower performance on the task in patients with schizophrenia than in control subjects.[43,44] Heritability was 0.53 (SE = 0.19).[33] Scores equal the number of words within the categories animals and professions generated in 60 seconds.

Early visual perception Backward Masking is a test of early visual information processing. More specifically, the location task taps activity of transient visual channels. In schizophrenia patients, performance is lower than in control subjects.[45] In our sample, heritability was 0.37 (SE = 0.17).[33] Backward masking procedures were performed using the computerised visual masking battery by Green et al.,[46] described in more detail previously.[33] Briefly, the backward masking paradigm involves a target (a square with an opening at one side) that is presented for 13.3 msec at the individual's critical stimulus intensity (determined in a no-mask condition), followed by a mask that consists of boxes occupying all possible target locations. The stimulus onset asynchrony (SOA) varied from 0 to 80 msec. Participants indicated where the target appeared. The test score was calculated as the arc sinus transformation of the mean percentage correct answers on the six trials with incrementing SOAs (13.3 to 80 ms). This value was multiplied by six to increase the standard deviation for univariate heritability analysis.

Spatial working memory The Spatial Span subtest of the Wechsler Memory Scale-III (WMS-III)[47] is a test of spatial working memory (SWM) and attention. Patients with schizophrenia show deficits, i.e., a lower score, on spatial span.[48] Heritability was in the high range (0.53, SE = 0.19).[33] The test involves increasing sequences of three-dimensional blocks pointed at by the

experimenter that are repeated by the participant in the same order (forward) or in reverse order (backward condition). The score equals the sum of correct repetitions in both conditions.

Intelligence. Four subtests of the Wechsler Adult Intelligence Scale-Third Edition (WAIS-III),[49] information, block design, arithmetic, and digit symbol substitution, were used to estimate the intelligence quotient (IQ). This combination of subtests has been shown to be the most reliable four-subtests version of estimating IQ in patients with schizophrenia,[50] on the basis of being not time-consuming and including one subtest of all four index scores of the WAIS-III. The sum of the four scaled subtest scores was multiplied by 11 and divided by 4, to make it equivalent to the 11-subtest sum. This score was then converted to the IQ score according to the WAIS-III manual.[49]

Data analysis

Standardised residuals corrected for age and sex were obtained from regression analysis and used for calculating Pearson's correlations and performing cluster analysis (using SPSS statistical software, version 12.0.2; SPSS Inc, Chicago, Illinois). Hierarchical agglomerative cluster analysis was performed on squared Euclidean distances (the sum of squared distances between variables) using the between-group linkage method, which averages the distance between all inter-cluster pairs. The program produces a dendrogram, in which the horizontal distance reflects similarity.

Bivariate heritability analyses were performed using the variance component-based program SOLAR, version 4.1 (Southwest Foundation for Biomedical Research, San Antonio, Texas).[51,52] Components of variance were estimated using the entire pedigrees by maximum likelihood including variation caused by significant covariates ($p < 0.1$) from the univariate analysis (as explained earlier)[33] in a multistep procedure. Univariate heritability of intelligence was estimated similarly using SOLAR. Covariates included were age for sensorimotor gating, openness, early visual perception, and spatial working memory; sex was included for openness alone. We did not include level of education in the analysis because, while it may be associated with the endophenotypes, it is also affected by schizophrenia. Bivariate heritability analysis determines to what extent the familial aggregation of a pair of traits may be attributed to shared genetic and environmental factors. We next estimated the phenotypic correlations (ρ_P) on the basis of bivariate heritability analyses, by taking the sum of the product of the genetic correlation (ρ_G) and the square roots of the genetic variances (h^2) of the two phenotypes and the product of the environmental correlation (ρ_E) and the square roots of the environmental variances ($1\text{-}h^2$) of the two phenotypes, as in: $\rho_P = \rho_G \cdot \sqrt{h_1^2} \cdot \sqrt{h_2^2} + \rho_E \cdot \sqrt{(1-h_1^2)} \cdot \sqrt{(1-h_2^2)}$, where subscript 1 and 2 refer to the two phenotypes.[53] The resulting estimated phenotypic correlations were compared with

Table 1 Descriptive statistics

Test	N (number of pedigrees)	M (± sd)	H^2 (± SE, p-value)
Intelligence	133 (24)	107.2 (± 14.5)	0.39 (± 0.162, 0.002)
Sensorimotor gating	131 (24)	63.8 (± 22.8)	0.46 (± 0.231, 0.009)[a]
Openness	163 (25)	39.2 (± 6.8)	0.54 (± 0.128, 0.000) [a]
Verbal Fluency	136 (25)	45.3 (± 9.3)	0.53 (± 0.192, 0.002) [a]
Early visual perception	124 (24)	0.54 (± 0.23)	0.37 (± 0.169, 0.005) [a]
Spatial working memory	144 (25)	16.3 (± 3.1)	0.53 (± 0.190, 0.001) [a]

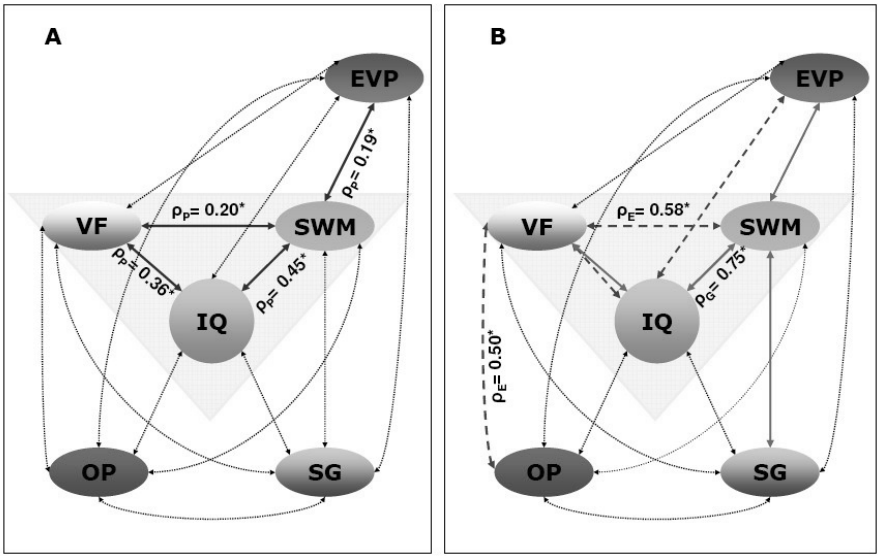
Note: Antipsychotic medication use was typical (6), atypical (25), both typical and atypical (2), none (4).

$H^{2:}$ heritability; M: mean;

[a] Estimated previously (Aukes ea, 2008).

the earlier mentioned calculated phenotypic correlations, as the latter ones were not corrected for relatedness of the participants, which could be a confounding factor. Both correlations were equivalent, implying that relatedness did not bias the correlation analyses. The squared genetic and environmental correlations give an estimate of the proportion of the variance attributable to

Figure 1 Simplified figural representations of Pearson's correlations (A) and genetic and environmental correlations (B)



Note: in A) significant Pearson's correlations and B) genetic (thick solid lines) and environmental (dashed lines) correlations above ±0.3 are given. The triangle indicates the main cluster from hierarchical cluster analysis.

EVP: early visual perception; IQ: intelligence quotient; OP: openness; SG: sensory gating; SWM: spatial working memory; VF: verbal fluency.

* Significant at $p < 0.05$.

Table 2 Pearson's correlations among the endophenotypes adjusted for age and sex

	SG	OP	VF	EVP	SWM
OP	-0.15 (129)				
VF	0.04 (126)	0.10 (135)			
EVP	-0.10 (117)	0.09 (122)	-0.07 (119)		
SWM	0.04 (131)	-0.13 (141)	0.20 (136) [a]	0.19 (124) [a]	
IQ	-0.04 (125)	0.14 (131)	0.36 (128) [b]	0.16 (120)	0.45(133) [b]

N given in parentheses. EVP: early visual perception; IQ: intelligence quotient; OP: openness; SG: sensory gating; SWM: spatial working memory; VF: verbal fluency.

[a] Significant at $p < 0.05$.

[b] Significant at $p < 0.01$.

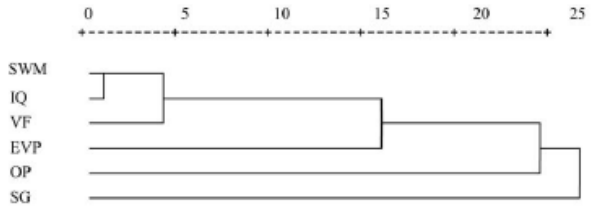
the additive effect of genes and environment, respectively. The significance of the genetic and environmental correlations were computed with a chi-square test by comparing the likelihoods of a polygenic model including the significant covariates to those of a model with the genetic or environmental correlations set at zero.

Results

Descriptive statistics are given in Table 1. Significant Pearson's correlations were found between spatial working memory and intelligence ($\rho = 0.45$, $p < 0.001$), verbal fluency and intelligence ($\rho = 0.36$, $p < 0.001$), verbal fluency and spatial working memory ($\rho = 0.20$, $p = 0.02$), and early visual perception and spatial working memory ($\rho = 0.19$, $p = 0.03$; Figure 1A and Table 2). The correlation between sensorimotor gating and openness showed a negative trend ($\rho = -0.15$, $p = 0.08$). The latter two did not show any significant correlations with other endophenotypes or with intelligence, suggesting these to be separate entities from the other endophenotypes and intelligence. Similarly, the cluster analysis first grouped spatial working memory and intelligence together, followed by verbal fluency. Subsequently, early visual perception was coupled to the cluster, then openness, and lastly sensorimotor gating (Figure 2).

The univariate heritability estimates of the endophenotypes have been reported previously (see Methods and Materials and Table 1).[33] The heritability for intelligence was estimated at 0.39 (SE = 0.16, $p = 0.002$). The variance component method is robust to violation of multivariate normality, though may be vulnerable to high levels of kurtosis in the trait distribution.[54] The levels of kurtosis in our sample were all within a range of -0.58 to 0.55; therefore, we did not transform the data.

Figure 2 Hierarchical cluster analysis: dendrogram showing the relative size of the proximity coefficients at which variables were combined



Note: the horizontal axis indicates the rescaled distance coefficients at which the clusters were combined. Highly similar variables are linked to each other on the left of the figure, indicating they were agglomerated into a cluster at a low distance coefficient.
EVP: early visual perception; IQ: intelligence quotient; OP: openness; SG: sensory gating; SWM: spatial working memory; VF: verbal fluency.

Bivariate heritability analysis quantifies the genetic and environmental components of the observed correlations between each pair of endophenotypes. Only the genetic correlation between spatial working memory and intelligence was significant ($\rho_G = 0.75$, SE = 0.19, p = 0.01; Figure 1B and Table 3). The model with the genetic correlation fixed at 1 could not be rejected (p = 0.06), meaning that these two traits may genetically overlap completely. The genetic contribution to the correlation between spatial working memory and intelligence was 75.6% (versus an environmental contribution of 24.4%) (Table 3). The squared genetic correlation is 0.57, which gives an indication of the proportion of genetic variance shared between the traits. The remaining part of the genetic variance (1-0.57) can be attributed to specific or independent genetic influences on the traits. Intelligence showed low genetic sharing with sensorimotor gating, openness, or early visual perception, and a higher, though not significant, genetic correlation with verbal fluency.
Significant environmental correlations were found between openness and verbal fluency ($\rho_E = 0.50$, SE = 0.19, p = 0.02) and verbal fluency and spatial working memory ($\rho_E = 0.58$, SE = 0.20, p = 0.02) (Figure 1B and Table 3). The shared environmental contribution to the correlation between openness and verbal fluency was 65.1%, and 77.3% for spatial working memory and verbal fluency (Table 3).

Discussion

This study investigated the genetic relationships among six promising endophenotypes, including intelligence, in pedigrees affected with schizophrenia. Five traits were selected from 13 candidate endophenotypes on the basis of heritability estimates of our earlier study,[33] including sensorimotor gating, openness, verbal fluency, early visual perception, and spatial working memory.

Table 3 Genetic (below diagonal) and environmental (above diagonal) correlations (± SE) among the endophenotypes with the proportion of respectively genetic (G) and environmental (E) contributions to the total phenotypic correlation in parentheses

	SG	OP	VF	EVP	SWM	IQ
SG		0.01 ± 0.23 (2.1%)	0.02 ± 0.30 (95.1%)	-0.05 ± 0.24 (26.0%)	-0.24 ± 0.25 (47.6%)	-0.12 ± 0.22 (63.0%)
OP	-0.29 ± 0.29 (97.9%)		**0.50 ± 0.19** [a] **(65.1%)**	0.14 ± 0.21 (59.8%)	0.02 ± 0.22 (5.4%)	0.17 ± 0.19 (72.0%)
VF	0.00 ± 0.39 (4.9%)	-0.26 ± 0.27 (34.9%)		-0.00 ± 0.24 (1.3%)	**0.58 ± 0.20** [a] **(77.3%)**	0.35 ± 0.18 (51.6%)
EVP	-0.18 ± 0.39 (74.0%)	0.11 ± 0.28 (40.2%)	-0.14 ± 0.41 (98.7%)		0.00 ± 0.25 (0.4%)	0.34 ± 0.18 (80.2%)
SWM	0.37 ± 0.51 (52.4%)	-0.26 ± 0.24 (94.6%)	-0.17 ± 0.34 (22.7%)	0.44 ± 0.28 (99.6%)		0.21 ± 0.20 (24.4%)
IQ	0.10 ± 0.38 (37.0%)	0.08 ± 0.27 (28.0%)	0.39 ± 0.27 (48.4%)	-0.15 ± 0.40 (19.8%)	**0.75 ± 0.20** [a] **(75.6%)**	

Note: G and E are calculated as the product of the genetic correlation (ρ_G) and the square roots of the genetic variances (h^2) of the two phenotypes and the product of the environmental correlation (ρ_E) and the square roots of the environmental variances ($1-h^2$) of the two phenotypes, respectively. Significant correlations are given in bold type.
E: environment; EVP: early visual perception; G: genetic; IQ: intelligence quotient; OP: openness; SG: sensory gating; SWM: spatial working memory; VF: verbal fluency.

[a] Significantly different from a model fixing the correlation at 0, at $p < 0.05$.

Our results demonstrate significant correlations among the cognitive endophenotypes spatial working memory, verbal fluency, and intelligence and between early visual perception and spatial working memory. The correlation between spatial working memory and intelligence can be mainly attributed to genetic factors (76%), expressed as a significant genetic correlation of 0.75. This genetic correlation implies that overlapping genetic effects contribute to individual differences in spatial working memory and intelligence. In contrast, two other candidate endophenotypes, i.e., sensorimotor gating and openness, appear to be separate heritable entities, sharing few genetic or environmental components with verbal fluency, spatial working memory, early visual perception, or intelligence. Sensorimotor gating and openness may thus be affected by separate genetic factors, possibly mediating distinct neurobiological mechanisms and different brain information processing functions. In other words, our results suggest that some cognitive endophenotypes, such as spatial working memory and intelligence, overlap genetically. These traits may therefore be combined in multivariate quantitative linkage and association studies to gain power in the search for the overlapping susceptibility loci for schizophrenia (e.g., by taking a sum or

factor score). On the other hand, endophenotypes such as sensorimotor gating and openness may identify distinct genetic variants contributing to schizophrenia.

The genetic correlation that we found between spatial working memory and intelligence is somewhat higher than estimates from previous studies in healthy twins.[17,18], though in line with expectations [26,31,55,56] The presumed higher frequency of susceptibility genes for schizophrenia in our multiply affected families as compared to healthy twins may explain the higher genetic correlation. The considerable genetic overlap among intelligence and a known cognitive endophenotype for schizophrenia, spatial working memory, supports the likelihood that intelligence is strongly related genetically with schizophrenia, as was reported by Toulopoulou et al.[21] This implies that genes related to intelligence may well overlap with genes that affect the vulnerability to develop schizophrenia.

Our findings also suggest that sensorimotor gating may be a construct genetically separate from the other endophenotypes tested. This observation agrees with the results of Greenwood et al.[15] showing few significant genetic or environmental correlations between sensorimotor gating and other cognitive endophenotypes for schizophrenia. Also in line with our results, phenotypic correlations have been low between sensorimotor gating and intelligence[57] and other psychophysiological measures.[58,59] Sensorimotor gating may thus represent a distinct genetic endophenotype that has potential to identify valuable genetic factors influencing a component of schizophrenia, which may not be detected by other (cognitive) endophenotypes.

We found no significant genetic correlations between openness and other endophenotypes for schizophrenia, including intelligence. This result supports the view of McCrae and Costa[60] that openness is not the same as intellect or cognitive ability, as was suggested by others.[60]. Although some have found openness to be the only one of the five personality factors to be positively related to measures of intelligence or cognition,[61,62] intelligence forms a distinct component when factored jointly with personality measures.[63] Therefore, our results suggest openness to be independent from the other measured endophenotypes for schizophrenia and may thus be influenced by distinct genetic factors for schizophrenia. Nevertheless, more research in relatives of patients with schizophrenia is needed to establish openness as an endophenotype for schizophrenia. Moreover, it would be worthwhile to investigate genetic overlap between openness and other personality, emotional, or social endophenotypes for schizophrenia. The only other measured personality endophenotype in our study, neuroticism, showed low genetic variation and was therefore not included in the bivariate heritability analysis.

The implication of our study that genes contributing to intelligence may strongly overlap with genes that influence other cognitive endophenotypes for schizophrenia and thus with schizophrenia itself is in line with the results of numerous genome-wide linkage scans. Suggestive linkage

peaks (logarithm of odds [LOD]>1) that are either overlapping or in close vicinity to each other have been found on chromosomes 1q, 2q, 6p, 7q, and 17q for intelligence[64-66] and schizophrenia.[67-81] Some of these regions have been linked to working memory as well.[11,80,82,83] Moreover, several candidate genes in these regions were found to influence cognitive traits, including working memory, and schizophrenia or both, e.g., ErbB4,[84] GAD1,[85] RELN,[80] PPP1R1B,[86] and Dysbindin.[87,88] These candidate genes are involved in gamma-aminobutyric acid (GABA) regulation,[89] neuronal migration (plasticity),[90] neuronal development,[91] and dopaminergic and glutamatergic pathways,[92-94] rendering these pathways into candidates for further exploration with regard to the relationship among intelligence, working memory, and schizophrenia.

The considerable number of genetic regions suggestively linked to intelligence, working memory, and schizophrenia, agrees with our previous finding that spatial working memory shows a complex or polygenic mode of transmission, i.e., is likely to be influenced by multiple genes.[33]. Such polygenic inheritance is also expected to apply to intelligence.[24] The findings of both multiple genetic influences and a high genetic correlation fit with the notion that both pleiotropy (in which one gene affects many traits) and heterogeneity (in which many genes affect a single trait) characterise common genes that affect general cognitive ability.[95] Interestingly, the two endophenotypes that show little genetic overlap with other endophenotypes, sensorimotor gating and openness, have previously shown a simpler Mendelian mode of inheritance[33] and are thus more likely to originate from distinct and fewer genetic factors. Our findings thus support the hypothesis that multiple genes have an effect on intelligence, related cognitive measures, and schizophrenia, while a smaller number of independent genetic factors may contribute to distinct endophenotypes, such as sensorimotor gating and openness. Despite the putative complex polygenic inheritance of intelligence, which diverges with criteria for endophenotypes, we believe intelligence can still be useful in unravelling the genetic components of schizophrenia. Its general endophenotypic characteristics, such as its quantitative nature, high heritabilility, reliability, and stability, and more specifically, its relative ease of administration in large numbers of samples and availability of such data, add to the strength of intelligence as a candidate endophenotype for schizophrenia.

The limited sample size of our bivariate analysis resulted in a wide range of standard errors. Nevertheless, we were able to delineate a pattern of genetic and environmental factors contributing to the endophenotypes that fits with theoretical pathogenic models and is congruent with other findings. Clearly, larger study samples are needed to further delineate the genetic structure underlying different sets of endophenotypes. A point of concern that remains is the correction for multiple testing (such as Bonferroni), which we did not apply since it assumes independency of traits and thus would be too strict.[96] Additionally, no ascertainment correction was performed, as

pedigrees were ascertained through disease status rather than task performance. One way to control for potentially biased scoring on endophenotypic tasks by patients is to perform the analysis without the patient data. Omitting the patient data in our analyses did not change the main results and conclusions of our study. The strength of our study is the detailed measurement of 13 endophenotypes in members of multiplex schizophrenic families of which we could select the most heritable ones. We combined different parallel analytic methods that yielded similar and coherent results.

In summary, we find strong correlations between intelligence and the cognitive endophenotypes for schizophrenia, spatial working memory, and verbal fluency. Most of the correlation between spatial working memory and intelligence can be explained by overlapping genetic factors. This suggests intelligence to be a promising endophenotype for genetic research in schizophrenia, even though the underlying genetic mechanism may still be complex. Two other promising endophenotypes for schizophrenia, sensorimotor gating and openness, appear to be separate entities with distinct but simpler genetic contributions. These endophenotypes are thus better candidates to find distinct molecular pathways involved in the aetiology of schizophrenia. We conclude that a balanced selection or combination of endophenotypes will improve the power to identify genes contributing to schizophrenia.

Acknowledgments

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References

1. Gottesman,II, Shields,J (1972): *Schizophrenia and Genetics; a Twin Study Vantage Point* . New York: Academic Press.
2. Walters JT, Owen MJ (2007): Endophenotypes in psychiatric genetics. *Mol Psychiatry* 12: 886-890.
3. Weeks DE, Lathrop GM (1995): Polygenic disease: methods for mapping complex disease traits. *Trends Genet* 11: 513-519.
4. Gottesman II, Gould TD (2003): The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 160: 636-645.
5. Heinrichs RW, Zakzanis KK (1998): Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. *Neuropsychology* 12: 426-445.
6. Sitskoorn MM, Aleman A, Ebisch SJ, Appels MC, Kahn RS (2004): Cognitive deficits in relatives of patients with schizophrenia: a meta-analysis. *Schizophr Res* 71: 285-295.

7. Tamminga CA, Holcomb HH (2005): Phenotype of schizophrenia: a review and formulation. *Mol Psychiatry* 10: 27-39.
8. Gur RE, Calkins ME, Gur RC, Horan WP, Nuechterlein KH, Seidman LJ et al (2007): The Consortium on the Genetics of Schizophrenia: neurocognitive endophenotypes. *Schizophr Bull* 33: 49-68.
9. Turetsky BI, Calkins ME, Light GA, Olincy A, Radant AD, Swerdlow NR (2007): Neurophysiological endophenotypes of schizophrenia: the viability of selected candidate measures. *Schizophr Bull* 33: 69-94.
10. Freedman R, Leonard S, Gault JM, Hopkins J, Cloninger CR, Kaufmann CA et al (2001): Linkage disequilibrium for schizophrenia at the chromosome 15q13-14 locus of the alpha7-nicotinic acetylcholine receptor subunit gene (CHRNA7). *Am J Med Genet* 105: 20-22.
11. Paunio T, Tuulio-Henriksson A, Hiekkalinna T, Perola M, Varilo T, Partonen T et al (2004): Search for cognitive trait components of schizophrenia reveals a locus for verbal learning and memory on 4q and for visual working memory on 2q. *Hum Mol Genet* 13: 1693-1702.
12. Ghosh S, Bhattacharjee S, Basu G, Pal S, Majumder PP (2005): Linkage mapping of a complex trait in the New York population of the GAW14 simulated dataset: a multivariate phenotype approach. *BMC Genet* 6 Suppl 1: S19.
13. Warren DM, Dyer TD, Peterson CP, Mahaney MC, Blangero J, Almasy L (2005): A comparison of univariate, bivariate, and trivariate whole-genome linkage screens of genetically correlated electrophysiological endophenotypes. *BMC Genet* 6 Suppl 1: S117.
14. Williams JT, Begleiter H, Porjesz B, Edenberg HJ, Foroud T, Reich T et al (1999): Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. II. Alcoholism and event-related potentials. *Am J Hum Genet* 65: 1148-1160.
15. Greenwood TA, Braff DL, Light GA, Cadenhead KS, Calkins ME, Dobie DJ et al (2007): Initial heritability analyses of endophenotypic measures for schizophrenia: the consortium on the genetics of schizophrenia. *Arch Gen Psychiatry* 64: 1242-1250.
16. Hall MH, Schulze K, Bramon E, Murray RM, Sham P, Rijsdijk F (2006): Genetic overlap between P300, P50, and duration mismatch negativity. *Am J Med Genet B Neuropsychiatr Genet* 141: 336-343.
17. Hansell NK, Wright MJ, Luciano M, Geffen GM, Geffen LB, Martin NG (2005): Genetic covariation between event-related potential (ERP) and behavioral non-ERP measures of working-memory, processing speed, and IQ. *Behav Genet* 35: 695-706.
18. Luciano M, Wright M, Smith GA, Geffen GM, Geffen LB, Martin NG (2001): Genetic covariance among measures of information processing speed, working memory, and IQ. *Behav Genet* 31: 581-592.
19. Posthuma D, de Geus EJ, Baare WF, Hulshoff Pol HE, Kahn RS, Boomsma DI (2002): The association between brain volume and intelligence is of genetic origin. *Nat Neurosci* 5: 83-84.
20. Reichenberg A, Weiser M, Rapp MA, Rabinowitz J, Caspi A, Schmeidler J et al (2005): Elaboration on premorbid intellectual performance in schizophrenia: premorbid intellectual decline and risk for schizophrenia. *Arch Gen Psychiatry* 62: 1297-1304.
21. Toulopoulou T, Picchioni M, Rijsdijk F, Hua-Hall M, Ettinger U, Sham P et al (2007): Substantial genetic overlap between neurocognition and schizophrenia: genetic modeling in twin samples. *Arch Gen Psychiatry* 64: 1348-1355.
22. Woodberry KA, Giuliano AJ, Seidman LJ (2008): Premorbid IQ in Schizophrenia: A Meta-Analytic Review. *Am J Psychiatry* 165: 579-87.
23. Bouchard TJ, Jr., McGue M (2003): Genetic and environmental influences on human psychological differences. *J Neurobiol* 54: 4-45.
24. Fisher PJ, Turic D, Williams NM, McGuffin P, Asherson P, Ball D et al (1999): DNA pooling identifies QTLs on chromosome 4 for general cognitive ability in children. *Hum Mol Genet* 8: 915-922.
25. Jensen AR (1998): *The g factor: the science of mental ability*. Westport, CT: Praeger Publishers.
26. Colom R, Rebollo I, Palacios A, Juan-Espinosa M, Kyllonen PC (2004): Working memory is (almost) perfectly predicted by g. *Intelligence* 32: 277-296.
27. Engle RW, Tuholski SW, Laughlin JE, Conway AR (1999): Working memory, short-term memory, and general fluid intelligence: a latent-variable approach. *J Exp Psychol Gen* 128: 309-331.

28. Alarcon M, Plomin R, Fulker DW, Corley R, DeFries JC (1998): Multivariate path analysis of specific cognitive abilities data at 12 years of age in the Colorado Adoption Project. *Behav Genet* 28: 255-264.
29. Pedersen NL, Plomin R, McClearn GE (1994): Is There G Beyond G - (Is There Genetic Influence on Specific Cognitive-Abilities Independent of Genetic Influence on General Cognitive-Ability. *Intelligence* 18: 133-143.
30. Petrill SA (1997): Molarity versus modularity of cognitive functioning? A behavioral genetic perspective. *Current Directions in Psychological Science* 6: 96-99.
31. Plomin R, Spinath FM (2002): Genetics and general cognitive ability (g). *Trends Cogn Sci* 6: 169-176.
32. Tsuang MT, Stone WS, Faraone SV (1999): The genetics of schizophrenia. *Curr Psychiatry Rep* 1: 20-24.
33. Aukes MF, Alizadeh BZ, Sitskoorn MM, Selten JP, Sinke RJ, Kemner C et al (2008): Finding Suitable Phenotypes for Genetic Studies of Schizophrenia: Heritability and Segregation Analysis. *Biol Psychiatry* 64: 128-36.
34. National Institute of Mental Health (1991): *Molecular Genetics Initiative: Family Interview for Genetic Studies*. Bethesda, MD, National Institute of Mental Health.
35. Andreasen NC, Flaum M, Arndt S (1992): The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnosis and psychopathology. *Arch Gen Psychiatry* 49: 615-623.
36. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E et al (1998): The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 59 Suppl 20: 22-33.
37. Braff DL, Geyer MA, Swerdlow NR (2001): Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)* 156: 234-258.
38. Bagby RM, Bindseil KD, Schuller DR, Rector NA, Young LT, Cooke RG et al (1997): Relationship between the five-factor model of personality and unipolar, bipolar and schizophrenic patients. *Psychiatry Res* 70: 83-94.
39. Camisa KM, Bockbrader MA, Lysaker P, Rae LL, Brenner CA, O'Donnell BF (2005): Personality traits in schizophrenia and related personality disorders. *Psychiatry Res* 133: 23-33.
40. Costa PT Jr., McCrae RR (1992): *Revised NEO Personality Inventory (NEO-PI-R) and NEO Five-Factor Inventory (NEO-FFI) professional manual*. Odessa, FL: Psychological Assessment Resources, Inc.
41. Hoekstra HA, Ormel J, de Fruyt F (1996): *NEO PI-R; NEO FFI; Big Five Persoonlijkheidsvragenlijsten; Handleiding*. Lisse: Swets & Zeitlinger B.V.
42. Luteijn F, Van der Ploeg F (1983): *The Groninger Intelligence Test (in Dutch)*. Lisse: Swets & Zeitlinger.
43. Bokat CE, Goldberg TE (2003): Letter and category fluency in schizophrenic patients: a meta-analysis. *Schizophr Res* 64: 73-78.
44. Henry JD, Crawford JR (2005): A meta-analytic review of verbal fluency deficits in schizophrenia relative to other neurocognitive deficits. *Cognit Neuropsychiatry* 10: 1-33.
45. Green MF, Nuechterlein KH, Breitmeyer B, Tsuang J, Mintz J (2003): Forward and backward visual masking in schizophrenia: influence of age. *Psychol Med* 33: 887-895.
46. Green MF, Nuechterlein KH, Breitmeyer B (2002): Development of a computerized assessment for visual masking. *Int J Methods Psychiatr Res* 11: 83-89.
47. Wechsler D (1997): *Wechsler Memory Scale,* 3rd ed. San Antonio, TX, the Psychological Corporation.
48. Lee J, Park S (2005): Working memory impairments in schizophrenia: a meta-analysis. *J Abnorm Psychol* 114: 599-611.
49. Wechsler D (2000): *WAIS-III Nederlandstalige Bewerking. Afname en scoringshandleiding*. Lisse, Swets Test Publishers.
50. Blyler CR, Gold JM, Iannone VN, Buchanan RW (2000): Short form of the WAIS-III for use with patients with schizophrenia. *Schizophr Res* 46: 209-215.
51. Almasy L, Dyer TD, Blangero J (1997): Bivariate quantitative trait linkage analysis: pleiotropy versus co-incident linkages. *Genet Epidemiol* 14: 953-958.

52. Almasy L, Blangero J (1998): Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 62: 1198-1211.
53. Lange K, Boehnke M (1983): Extensions to pedigree analysis. IV. Covariance components models for multivariate traits. *Am J Med Genet* 14: 513-524.
54. Allison DB, Neale MC, Zannolli R, Schork NJ, Amos CI, Blangero J (1999): Testing the robustness of the likelihood-ratio test in a variance-component quantitative-trait loci-mapping procedure. *Am J Hum Genet* 65: 531-544.
55. Donohoe G, Clarke S, Morris D, Nangle JM, Schwaiger S, Gill M et al (2006): Are deficits in executive sub-processes simply reflecting more general cognitive decline in schizophrenia? *Schizophr Res* 85: 168-173.
56. Kyllonen PC, Christal RE (1990): Reasoning Ability Is (Little More Than) Working-Memory Capacity. *Intelligence* 14: 389-433.
57. Ornitz EM, Russell AT, Gabikian P, Gehricke JG, Guthrie D (2000): Prepulse inhibition of startle, intelligence and familial primary nocturnal enuresis. *Acta Paediatr* 89: 475-481.
58. Braff DL, Light GA, Swerdlow NR (2007): Prepulse inhibition and P50 suppression are both deficient but not correlated in schizophrenia patients. *Biol Psychiatry* 61: 1204-1207.
59. Oranje B, van Berckel BN, Kemner C, van Ree JM, Kahn RS, Verbaten MN (1999): P50 suppression and prepulse inhibition of the startle reflex in humans: a correlational study. *Biol Psychiatry* 45: 883-890.
60. McCrae RR, Costa PT, Jr. (1997): Conceptions and correlates of openness to experience. In Hogan R, Johnson J, Briggs SR, editors. *Handbook of Personality Psychology*. San Diego: Academic Press, pp 825-847.
61. Saggino A, Balsamo M (2003): Relationship between WAIS-R intelligence and the five-factor model of personality in a normal elderly sample. *Psychol Rep* 92: 1151-1161.
62. Miller GF, Tal IR (2007): Schizotypy versus openness and intelligence as predictors of creativity. *Schizophr Res* 93: 317-324.
63. McCrae RR (1994): Openness to Experience - Expanding the Boundaries of Factor-V. *European Journal of Personality* 8: 251-272.
64. Posthuma D, Luciano M, Geus EJ, Wright MJ, Slagboom PE, Montgomery GW et al (2005): A genomewide scan for intelligence identifies quantitative trait loci on 2q and 6p. *Am J Hum Genet* 77: 318-326.
65. Dick DM, Aliev F, Bierut L, Goate A, Rice J, Hinrichs A et al (2006): Linkage analyses of IQ in the collaborative study on the genetics of alcoholism (COGA) sample. *Behav Genet* 36: 77-86.
66. Luciano M, Wright MJ, Duffy DL, Wainwright MA, Zhu G, Evans DM et al (2006): Genome-wide scan of IQ finds significant linkage to a quantitative trait locus on 2q. *Behav Genet* 36: 45-55.
67. Antonarakis SE, Blouin JL, Pulver AE, Wolyniec P, Lasseter VK, Nestadt G et al (1995): Schizophrenia susceptibility and chromosome 6p24-22. *Nat Genet* 11: 235-236.
68. Bailer U, Leisch F, Meszaros K, Lenzinger E, Willinger U, Strobl R et al (2000): Genome scan for susceptibility loci for schizophrenia. *Neuropsychobiology* 42: 175-182.
69. Ekelund J, Lichtermann D, Hovatta I, Ellonen P, Suvisaari J, Terwilliger JD et al (2000): Genome-wide scan for schizophrenia in the Finnish population: evidence for a locus on chromosome 7q22. *Hum Mol Genet* 9: 1049-1057.
70. Hennah W, Tomppo L, Hiekkalinna T, Palo OM, Kilpinen H, Ekelund J et al (2007): Families with the risk allele of DISC1 reveal a link between schizophrenia and another component of the same molecular pathway, NDE1. *Hum Mol Genet* 16: 453-462.
71. Hovatta I, Lichtermann D, Juvonen H, Suvisaari J, Terwilliger JD, Arajarvi R et al (1998): Linkage analysis of putative schizophrenia gene candidate regions on chromosomes 3p, 5q, 6p, 8p, 20p and 22q in a population-based sampled Finnish family set. *Mol Psychiatry* 3: 452-457.
72. Klei L, Bacanu SA, Myles-Worsley M, Galke B, Xie W, Tiobech J et al (2005): Linkage analysis of a completely ascertained sample of familial schizophrenics and bipolars from Palau, Micronesia. *Hum Genet* 117: 349-356.
73. Lerer B, Segman RH, Hamdan A, Kanyas K, Karni O, Kohn Y et al (2003): Genome scan of Arab Israeli families maps a schizophrenia susceptibility gene to chromosome 6q23 and supports a locus at chromosome 10q24. *Mol Psychiatry* 8: 488-498.

74. Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I et al (2003): Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 73: 34-48.
75. Lindholm E, Ekholm B, Balciuniene J, Johansson G, Castensson A, Koisti M et al (1999): Linkage analysis of a large Swedish kindred provides further support for a susceptibility locus for schizophrenia on chromosome 6p23. *Am J Med Genet* 88: 369-377.
76. Maziade M, Roy MA, Rouillard E, Bissonnette L, Fournier JP, Roy A et al (2001): A search for specific and common susceptibility loci for schizophrenia and bipolar disorder: a linkage study in 13 target chromosomes. *Mol Psychiatry* 6: 684-693.
77. Paunio T, Ekelund J, Varilo T, Parker A, Hovatta I, Turunen JA et al (2001): Genome-wide scan in a nationwide study sample of schizophrenia families in Finland reveals susceptibility loci on chromosomes 2q and 5q. *Hum Mol Genet* 10: 3037-3048.
78. Schwab SG, Hallmayer J, Albus M, Lerer B, Eckstein GN, Borrmann M et al (2000): A genome-wide autosomal screen for schizophrenia susceptibility loci in 71 families with affected siblings: support for loci on chromosome 10p and 6. *Mol Psychiatry* 5: 638-649.
79. Straub RE, MacLean CJ, Ma Y, Webb BT, Myakishev MV, Harris-Kerr C et al (2002): Genome-wide scans of three independent sets of 90 Irish multiplex schizophrenia families and follow-up of selected regions in all families provides evidence for multiple susceptibility genes. *Mol Psychiatry* 7: 542-559.
80. Wedenoja J, Loukola A, Tuulio-Henriksson A, Paunio T, Ekelund J, Silander K et al (2007): Replication of linkage on chromosome 7q22 and association of the regional Reelin gene with working memory in schizophrenia families. *Mol Psychiatry 13: 673-84*.
81. Wijsman EM, Rosenthal EA, Hall D, Blundell ML, Sobin C, Heath SC et al (2003): Genome-wide scan in a large complex pedigree with predominantly male schizophrenics from the island of Kosrae: evidence for linkage to chromosome 2q. *Mol Psychiatry* 8: 695-705, 643.
82. Hansell NK, Medland SE, Ferreira MA, Geffen GM, Zhu G, Montgomery GW et al (2006): Linkage analyses of event-related potential slow wave phenotypes recorded in a working memory task. *Behav Genet* 36: 29-44.
83. Hennah W, Tuulio-Henriksson A, Paunio T, Ekelund J, Varilo T, Partonen T et al (2005): A haplotype within the DISC1 gene is associated with visual memory functions in families with a high density of schizophrenia. *Mol Psychiatry* 10: 1097-1103.
84. Nicodemus KK, Luna A, Vakkalanka R, Goldberg T, Egan M, Straub RE et al (2006): Further evidence for association between ErbB4 and schizophrenia and influence on cognitive intermediate phenotypes in healthy controls. *Mol Psychiatry* 11: 1062-1065.
85. Straub RE, Lipska BK, Egan MF, Goldberg TE, Callicott JH, Mayhew MB et al (2007): Allelic variation in GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. *Mol Psychiatry* 12: 854-869.
86. Meyer-Lindenberg A, Straub RE, Lipska BK, Verchinski BA, Goldberg T, Callicott JH et al (2007): Genetic evidence implicating DARPP-32 in human frontostriatal structure, function, and cognition. *J Clin Invest* 117: 672-682.
87. Burdick KE, Lencz T, Funke B, Finn CT, Szeszko PR, Kane JM et al (2006): Genetic variation in DTNBP1 influences general cognitive ability. *Hum Mol Genet* 15: 1563-1568.
88. Donohoe G, Morris DW, Clarke S, McGhee KA, Schwaiger S, Nangle JM et al (2007): Variance in neurocognitive performance is associated with dysbindin-1 in schizophrenia: a preliminary study. *Neuropsychologia* 45: 454-458.
89. Pinal CS, Tobin AJ (1998): Uniqueness and redundancy in GABA production. *Perspect Dev Neurobiol* 5: 109-118.
90. Fatemi SH (2005): Reelin glycoprotein: structure, biology and roles in health and disease. *Mol Psychiatry* 10: 251-257.
91. Huang YZ, Won S, Ali DW, Wang Q, Tanowitz M, Du QS et al (2000): Regulation of neuregulin signaling by PSD-95 interacting with ErbB4 at CNS synapses. *Neuron* 26: 443-455.
92. Halpain S, Girault JA, Greengard P (1990): Activation of NMDA receptors induces dephosphorylation of DARPP-32 in rat striatal slices. *Nature* 343: 369-372.
93. Talbot K, Eidem WL, Tinsley CL, Benson MA, Thompson EW, Smith RJ et al (2004): Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. *J Clin Invest* 113: 1353-1363.

94. Numakawa T, Yagasaki Y, Ishimoto T, Okada T, Suzuki T, Iwata N et al (2004): Evidence of novel neuronal functions of dysbindin, a susceptibility gene for schizophrenia. *Hum Mol Genet* 13: 2699-2708.
95. Kovas Y, Plomin R (2006): Generalist genes: implications for the cognitive sciences. *Trends Cogn Sci* 10: 198-203.
96. Rothman KJ (1990): No adjustments are needed for multiple comparisons. *Epidemiology* 1: 43-46.

Chapter 4

A Genome-wide Linkage Study of Cognitive Endophenotypes for Schizophrenia highlights the Neurotrophin receptor gene (NTRK3) as a Susceptibility Locus

Submitted

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Abstract

We performed a genome-wide linkage scan of six schizophrenia endophenotypes with moderate heritability: sensorimotor gating, openness, verbal fluency, early visual perception, spatial working memory, and intelligence. The study included seven extended multiply affected Dutch pedigrees (n=118, 649 relative pairs) for which data of 6,090 single nucleotide polymorphisms (SNPs) genome-wide was collected. Two-point and multipoint variance-component based linkage analyses were performed using MERLIN.

Multipoint-linkage peaks with suggestive evidence were detected at 8q24 for early visual perception (LOD= 1.8) and 17p13 and 16q21-22 for openness (LOD= 1.7 and 1.5). Suggestive two-point hits (empirical p-value<0.001) were observed for sensorimotor gating, verbal fluency, spatial working memory, and IQ in several regions, including 2q22.1, 5q33-34, 9q31-33, and 15q26.1. Assuming a genetic relationship between endophenotypes and schizophrenia we performed subsequent prioritization analyses of positional candidate genes for schizophrenia followed by reviewing expression in brain tissues of schizophrenia patients. Our efforts highlighted NTRK3 located at chromosome 15q26.1. Subsequent analyses in available case/control cohorts yielded a consistent association signal with schizophrenia in a Dutch sample of 758/676 cases/controls (p=0.0005) as well as in two other independent (GAIN) datasets (p=0.00006 and 0.0004), supporting the involvement of NTRK3 in schizophrenia.

This study provides the first genome-wide scan for sensorimotor gating, openness, and verbal fluency. We identified several candidate loci with suggestive evidence, and highlight in particular the NTRK3 gene involvement in spatial working memory and schizophrenia susceptibility.

Introduction

Schizophrenia (MIM# 181500) is a complex and chronic disorder that affects approximately 1% of the population.[1] Although it has a heritability of up to 80%,[2] the inheritance of schizophrenia is complex in nature, involving multiple genes of limited effect, interacting with environmental factors.[2,3] Thus far, linkage and association studies have pinpointed several genomic regions and candidate genes for schizophrenia. However, few findings have been consistently replicated, the most significant linked regions being 2q, 5q, and 8p.[4-6]

Schizophrenia is a broad heterogeneous diagnostic entity which is clinically relevant, though less appropriate for etiological and genetic research. Endophenotypes refer to heritable traits associated with an increased risk for schizophrenia[7] that may aid in localizing genetic variants underlying schizophrenia by having characteristics such as a reduced phenotypic heterogeneity. A promising candidate endophenotype for schizophrenia is cognition, as for a wide range of cognitive traits it has been shown that patients with schizophrenia exhibit deviant behavior before the onset of illness[8] and beyond;[9] are deviant in the unaffected relatives,[10] and heritable.[11]

Despite the potential of cognitive endophenotypes, few genetic studies have incorporated cognitive endophenotypes for schizophrenia into linkage analysis. These few studies have revealed several candidate susceptibility loci for endophenotypes, e.g., 6p21-23, 15q14, 22q11-q12, and 4q21, though most of the findings await replication.[12-23] To our knowledge, no genome wide linkage scan has been performed previously for sensorimotor gating, openness, verbal fluency, and early visual perception (backward masking).

We have previously investigated several endophenotypes for degree of familial correlations, proportion of heritability, patterns of inheritance,[11] and degree of genetic correlations among these endophenotypes.[24] On the basis of these parameters, we have selected several heritable candidate endophenotypes for schizophrenia including sensorimotor gating, openness, verbal fluency, early visual perception, spatial working memory, and IQ, as suitable traits for genetic investigations. In the present study, our aim was to identify quantitative trait loci (QTLs) that influence variation in these endophenotypes by means of a genome-wide linkage analysis in seven Dutch multigenerational families with at least one subject affected with schizophrenia.

Materials and Methods

A figure displaying the study design is provided in Supplementary Figure S1.

Participants Seven pedigrees including 118 individuals (Supplementary Figure S2) were selected on the basis of size and information content (using SLINK)[25] from a larger sample of 25

multiplex families that has been described previously.[11,24] The average size of the families included in the linkage study was roughly 17 members (11 to 26) distributed over three generations or more (3: 71.4%, 4: 28.6%). The pedigrees encompass 649 relative pairs, including 85 parent-offspring, 117 sibling, 144 avuncular, 104 first cousin, and 49 first cousins once removed pairs, as well as several grandparent-grandchild, half-sibling, half-avuncular, half-cousin, grand-avuncular, second cousins, and unrelated pairs. No loops or consanguineous mating pairs were present. All families were of Dutch origin and were ascertained by means of a family-member society and newspaper advertisements. Each family comprised at least two members with a schizophrenia or schizoaffective disorder diagnosis of which at least one member's diagnosis was confirmed. We screened the families for the presence of diagnoses of schizophrenia or related disorders using the Family Interview for Genetic Studies (FIGS)[26] conducted by telephone with a family member. Patients were diagnosed by DSM-IV criteria on the basis of the Comprehensive Assessment of Symptoms and History (CASH), a semi-structured diagnostic interview[27] and by retrieving medical records. Psychiatric illness in relatives was assessed by means of the Mini International Neuropsychiatric Interview (MINI-PLUS), a structured clinical interview of DSM axis 1 diagnoses.[28]

Exclusion criteria for all participants were: severe medical or neurological illness; history of closed-head injury; loss of consciousness longer than 30 minutes; history of alcohol abuse within last 6 months; diseases of the central nervous system and history of cerebrovascular accidents, dementia or delirium; age < 16; or IQ < 70. All patients used antipsychotic medication, either typical (n=3), atypical (n=9), or both (n=1). Written informed consent was obtained from all participants after complete description of the study. The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht.

Endophenotyping We previously performed extensive analyses and selected the most promising endophenotypes for this linkage study.[11,24] From 13 candidate endophenotypes, which were selected based on satisfying most or all criteria for endophenotypes,[7] five traits fulfilled our selection criterion of moderate familial correlation i.e., sensorimotor gating, openness, verbal fluency, early visual perception, and spatial working memory.[11] Heritability estimates were equivalent (37%-54%).[11]

All tasks have been described in more detail previously.[11] Briefly, we measured *sensorimotor gating* (SG) using the prepulse inhibition (PPI) task; *openness to experience* (OP) using the Neuroticism-Extroversion-Openness - Five Factor Inventory (NEO-FFI);[29] *verbal fluency* (VF) using the Category Fluency task;[30] *early visual perception* (EVP) using the location condition of the Backward Masking task;[31] s*patial working memory* (SWM) using the Spatial Span subtest of the

Wechsler Memory Scale-III (WMS-III);[32] and *intelligence quotient* (IQ) using four subtests of the Wechsler Adult Intelligence Scale (WAIS-III).[33]

Genotyping Genomic DNA was extracted from EDTA blood samples using standard salting out procedure. We typed 6,090 SNPs distributed evenly across the genome using Illumina Infinium HumanLinkage-12 arrays (Illumina, San Diego, CA). The mean and median interval between markers is 0.44 Mb (0.58 cM) and 0.32 Mb (0.35 cM), respectively. The SNPs were genotyped using the Illumina BeadArray™ technology on an Illumina BeadStation following the manufacturer's protocol.

Error detection All SNPs were examined for their resulting quality; SNPs with a low signal or incomplete clusters were excluded (n=80). PLINK (version 1.05)[34] was used to check the data for gender errors (none). We checked for Mendelian inconsistencies using the program Pedcheck[35] and identified problem genotypes using MERLIN.[36] Sporadic genotyping errors (n = 38) were removed within families. One family showed segregation errors as well as deviations on two measures of familial relatedness calculated in PLINK: identity by state (IBS) distance (= (IBS2 + 0.5*IBS1)/N SNP pairs) and proportion of identity by descent (IBD) (= P (IBD=2) +0.5*P(IBD=1). Supplementary Figure S3 shows the calculated relatedness plotted against the expected relationships. We found the results to be consistent with non-paternity and a dummy father was introduced for one of three sibs. Additionally, we identified a non-affected individual with a maternally inherited uniparental disomy (UPD) of chromosome 22. Previous reports suggest that maternal UPD 22 has no effect on the phenotype,[37] which was in agreement with our observations. This individual was removed from the analysis of chromosome 22 only.

Data analysis Descriptive statistics were calculated using the program Pedstats (version 0.6.10).[38] Non-parametric univariate two-point and multipoint linkage analyses were performed by the means of variance-components (VC) based models implemented in MERLIN, and MINX[36] for the X chromosome. VC linkage analysis estimates the proportion of variance that can be explained by an underlying QTL, by examining the expected genetic covariances between relatives as a function of their IBD relationships at a given SNP.

In MERLIN, IBD is calculated using the Lander-Green algorithm with sparse gene flow trees and background covariance is assumed to be entirely due to additive genetic effects. Sex and age were included as covariates. The null hypothesis that the additive genetic variance in a trait caused by a QTL linked to a given SNP is zero was tested by comparing the likelihood of a reduced model in which σ_q^2 was constrained to zero (i.e., $\sigma_q^2 = 0$) with the likelihood of a model in which the genetic variance due to the QTL was estimated. Twice the difference in natural log-likelihood between these models is distributed as χ^2, whereas the difference between the two $\log_{10}$ likelihoods produces a LOD score equivalent to the classical LOD score of parametric link-

age analysis. In multipoint analysis, we used two centimorgan (cM) spacing. While VC based methods depend on trait normality, the method is quite robust to distributional violations, except for high kurtosis.[39] Since the kurtosis of each of our endophenotypes was smaller than 1, we did not transform our data. We performed sensitivity analyses to insure that the peak LOD scores were not due to the effect of a single family.

Simulation analysis The sample size is of the current study is relatively small which results in limited statistical power for detecting linkage. We performed a simulation using SLINK and SOLAR which showed that the power for detecting suggestive linkage with LOD<2 was less than 50% for all traits. Moreover since familial relationships are not uniformly distributed in our pedigree sample (as in sibling pairs), it is recommended to empirically estimate genome-wide significance levels. It protects against false positives, and potentially enhances the power of the analysis by adjusting for possible biases induced by factors such as outliers or non-random missing data. We performed two-point linkage analyses on 1,000 simulated data sets. For each simulation, new genotypes were simulated with MERLIN under the null hypothesis of no linkage, while phenotypic data, allele frequencies, marker spacing, and missing data pattern were kept the same. For each of the findings we calculated empirical point-wise p-values by counting the proportion of genome scans containing one or more peaks of that size. Thresholds for suggestive (once in a genome-wide scan) and significant linkage (once in 20 genome scans) for each trait were, respectively: OP: 1.64, 2.37; SG: 1.68 2.40; VF: 1.66, 2.36; EVP6: 1.75, 2.54; SWM: 1.40, 2.40; IQ: 1.37, 1.96. For multipoint linkage simulation we applied the thresholds for suggestive and significant LOD scores of 1.9 and 3.3 as suggested by Lander and Kruglyak.[40]

Prioritization If we assume endophenotypes are genetically related to schizophrenia, we may search for candidate genes for schizophrenia that reside in the linkage regions by investigating gene networks in a prioritization analysis. Prioritization involves extracting information from public online databases, such as sequence data, medical literature, gene ontology, function annotation, and gene expression for all genes in the regions of interest. We used two different methods of prioritization: Prioritizer (v1.2),[41] a hypothesis free method, and Endeavour (web version 2009),[42] which uses a training set of genes known to be involved in schizophrenia. The training set in Endeavour included the 26 highest ranked candidate genes for schizophrenia (Supplemental Table S1; obtained from the SZgene website,[43] see Web resources, a large meta-analysis of association analyses for schizophrenia; accessed April 2009), excluding candidate genes (GABRB2 and TP53) that were located within the defined regions when analyzing these regions. We applied regions of 1 Mb surrounding the suggestive two-point SNPs and 2, 8, 3, and 4 Mb surrounding multipoint linkage peaks on 2p, 8q, 16q and 17p, respectively, and analyzed these

for each trait separately and for combinations of two traits if traits were genetically correlated (>0.3).[24]

Expression We selected the best candidate genes which i) were prioritized at a statistical significance level at a p<0.05 or integrated in a direct interaction network in Prioritizer and ii) were prioritized at a significance level of p<0.05 in Endeavour (Supplementary Table S2). We examined the levels of expression of the prioritized genes (n=8) in two recently published samples of the prefrontal cortex in schizophrenia compared to carefully matched control subjects ($n_{affected/unaffected}$ = 28/23 and 16/27 [44,45] and in online samples from the cerebellar cortex. The cerebellar cortex data from schizophrenia patients and sex-, age-, and post-mortem interval (PMI)-matched control subjects (GEO accession GDS1917, see Web resources) were analyzed using the Mann-Whitney U test. There were no significant differences in sex, age, or PMI between the groups.

Association analysis We defined potential candidates genes for schizophrenia as those which were i) under linkage peaks, and ii) prioritized with sufficient statistical significance (see above) and iii) showed a significant differential expression in patients with schizophrenia compared to control subjects at level of p<0.05. In total, four genes met our criteria. A two-staged association analysis was performed. In the screening stage, these candidate genes were screened for an association with schizophrenia in a set of 758 schizophrenia cases and 676 control subjects from the Netherlands, who had already been genotyped using the genome-wide Illumina 550K array (Illumina, San Diego, CA). In the replication stage, we examined the best candidate gene (p<0.001) using two GAIN sample sets including 1,172/1,378 and 921/954 cases/controls (see Web re

Table 1 Sample characteristics of the linkage sample

	N	Mean ± sd (range)	Sibling correlation	Heritability[a]
Total	118	-	-	-
Genotyped	91	-	-	-
Age	-	44.4 ± 17.3 (16 - 81)	-	-
Sex (% male)	58	-	-	-
Sensorimotor gating	67	67.5 ± 22.6 (3.3 - 97.1)	0.05	39.53
Openness	83	37.9 ± 6.9 (22 - 56)	0.28	42.31
Verbal fluency	68	45.5 ± 9.3 (25 - 68)	0.30	81.43
Early visual perception	62	3.5 ± 1.4 (1.3 - 6.8)	0.53	35.42
Spatial working memory	72	16.7 ± 3.1 (11 - 24)	0.37	26.68
IQ	68	108.6 ± 15.0 (76 - 144)	0.20	37.66

Abbreviations: sd: standard deviation.

[a] Representing heritability estimates for the current study sample, which may therefore deviate from our earlier reported estimates in a larger sample[11].

sources) that were genotyped genome-wide using the Affymetrix 6.0 array. Association analyses were performed using model-based association testing implemented in PLINK followed by ten thousand permutations to address multiple testing.

Table 2 Two-point linkage results with a LOD >1.5

Trait	Cytogenetic Band	Marker	Map distance [a]	LOD	Empirical p	Pedigrees [b]
Sensorimotor gating	5q33.3-34	**rs728693**	**162**	**2.14**	**0.00015**	All
		rs13178296	**165**	**2.11**	**0.00017**	2,3,4,7
	7p21.1	rs1723804	30	1.56	0.00172	2,5,6
	11p15.3	rs16908224	17	1.54	0.00183	1,2,5,6,7
	17q22-23.2	rs792786	80	1.56	0.00171	1,2,5,6,7
		rs929648	87	1.54	0.00184	1,2,4,5,6
Openness	7q22.3-31.33	rs234	115	1.60	0.00119	1,2,3,4,5
		rs1419607	128	1.50	0.00173	1,2,4,5
	17p13.1	rs7221818	15	1.54	0.00150	1,3,4,5,6
Verbal fluency	1q22	rs1194580	147	1.66	0.00100	1,2,5,6,7
		rs2066981	**148**	**1.79**	**0.00060**	1,4,5,6,7
	2q37.1	rs7286	240	1.65	0.00104	2,3,5,6,7
		rs838715	**241**	**1.70**	**0.00085**	2,3,4,5,6
	9q31.2-33.1	**rs1516882**	**109**	**1.71**	**0.00082**	1,2,3,4,6
		rs1407850	**112**	**2.04**	**0.00020**	1,2,4,5,6,7
		rs10081701	**118**	**2.54**	**0.00002**	2,3,5,6,7
	10p13	**rs1892302**	**30**	**1.67**	**0.00097**	3,5,6
		rs652029	32	1.59	0.00136	2,3,5,6,7
		rs4615920	**33**	**1.88**	**0.00041**	1,2,3,5,6,7
	11q24.3-25	rs1944142	144	1.51	0.00187	2,3,5,6
	12p13.33	rs2107614	1	1.64	0.00108	2,3,5,6
		rs1005394	2	1.58	0.00138	2,3,5,6,7
		rs766956	2	1.58	0.00139	2,3,5,6,7
	12q24.23	**rs1016203**	**138**	**1.69**	**0.00088**	1,2,3,5,6,7
Early visual perception	20p13	rs12624577	3	1.71	0.00116	1,2,4,5,6,7
Spatial working memory	15q26.1	**rs8025499**	**94**	**1.68**	**0.00044**	1,2,3,4,5,7
	Xp22.11	**rs739974**	**41**	**1.98**	**0.00018**	2,4,5,6,7
	Xq21.31-22.1	**rs2030392**	**96**	**1.70**	**0.00042**	All
		rs761843	**104**	**1.92**	**0.00022**	1,2,3,5,6,7
IQ	2q22.1	**rs1918615**	**152**	**1.78**	**0.00013**	All

Note: Bolded are findings that cross the conventional p-value threshold for suggestive linkage of 0.0001.

[a] Map distance in cM corresponds with the deCode genetic map, sex-averaged distances.

[b] Pedigrees contributing to the LOD score.

Table 3 Multipoint linkage results with a LOD>1.5

Trait	Cytogenetic Band	Map distance [a] (LOD>1 region)	LOD	P-value	Pedigrees[b]
Openness	16q21-22.1	83 (81-85)	1.53	0.0040	1,2,4,5,6
	17p13.2-13.1	15 (15-17)	1.70	0.0026	1,3,5,6
Early visual perception	2p21	72	1.50	0.0043	1,2,3,4,5
	8q24.2-24.3	152 (146-168)	1.77	0.0022	1,2,3,4,5,7

[a] Map distance in cM corresponds with the deCode genetic map, sex-averaged distances. In parentheses the boundaries of the linkage peak (LOD>1)

[b] Pedigrees contributing to the LOD score.

Results

Sample characteristics and a description of phenotypic data are provided in Table 1. The average marker heterozygosity was 44.5% in our sample. The average minor allele frequency was 35% in the founders. The missing rate was 0.014%. The mean genome-wide information content, over all chromosomes, was 0.89 (range: 0.65-0.98; standard deviation (sd) = 0.03; two-point average= 0.31, range= 0.01-0.55). Fifteen markers in 10 genomic regions passed the threshold for suggestive linkage in two-point analysis (empirical p-value<0.001; Table 2; Supplementary Figure S4), whereas no genomic regions passed the genome-wide threshold for suggestive or significant multipoint linkage analysis (Table 3; Supplementary Figure S5). Below, we present results by endophenotype.

Sensorimotor gating Two-point linkage resulted in two linkage peaks for the rs728693 and rs13178296 SNPs mapped to 5q33-34 that passed the suggestive empirical threshold for sensorimotor gating (both LOD = 2.1, empirical $p \leq 0.00017$). In multipoint linkage analysis we did not obtain LOD-scores higher than 1 for sensorimotor gating (Supplementary Figure S5). When we performed a prioritization analysis of the 5q33-34 region, the Gamma-aminobutyric-acid (GABA(A) receptor subunit alpha-1 gene [GABRA1], MIM#137160) and the ADAM19 precursor gene (ADAM19, MIM#603640) near SNPs rs13178296 and rs728693, respectively, were prioritized for schizophrenia based on their functional interconnections with candidate genes for schizophrenia. However, these genes did not fulfill our criterion of being highlighted in both prioritization methods; therefore we did not examine their expression levels in the brain of schizophrenia patients versus unaffected controls.

Table 4 Linkage, prioritization, expression, and association findings support NTRK3 as a positional candidate gene

	Linkage Endophenotype				Pathway				Function	Genetic	
Trait	Cytogenetic Band	Marker	Map distance [a]	LOD	Candidate Gene	Prioritizer result	Endeavour p-value	Trait(s)[b]	Expression SCZ brain p-value [c]	SCZ Association [d]	SCZ Association replication samples [e]
Verbal fluency	2q37.1	rs7286	240	1.65	EIF4E2	snw	0.03	VF, VF_IQ	0.047 [f]	0.002	
					GIGYF2	na	0.007	VF, VF_IQ	0.01 [f]	ns	
	9q31.2-33.1	rs10081701	118	2.54	RGS3	na	0.008	VF, VF_IQ	0.01 [g]	ns	
Spatial working memory	15q26.1	rs8025499	94	1.68	NTRK3	snw	0.002	SWM_EVP	0.039 [g] 0.009 [f]	0.0006	$6.4*10^{-5}$

Note: details for all prioritized genes are provided in supplementary Table S3.

Abbreviations: na: not available; snw: small network in Prioritizer, SCZ: schizophrenia; MP: multipoint.

[a] Map distance in cM corresponds with the deCode genetic map, sex-averaged distances.

[b] The trait or combination of traits for which regions were analyzed in prioritization analysis. We did not perform analyses on the single regions for SG and IQ separately.

[c] A dash indicates that data was not available for HBB and CCHPC samples or not significant. Details for the GDS1917-study are given in supplementary Table S2.

[d] Best p-values are shown. Association analyses were performed in 758 cases compared to 676 control subjects. If empty, association analysis was not performed (see text).

[e] Best p-value is shown. Association analyses were performed in two replication samples of 1,172/1,378 and 921/954 schizophrenia cases/controls (GAIN), respectively. If empty, association analysis was not performed (see text).

[f] GEO accession GDS1917, see Web resources.

[g] Charing Cross Hospital prospective collection[45].

Openness In total, three SNPs showed a LOD higher than 1.5 for openness in the two-point analysis; at 7q22.3-31.33 (rs234 and rs149607) and 17p13.1 (rs7221818). In multipoint linkage analysis, we observed a linkage peak for the same 17p13 region (at 15 cM) with a LOD of 1.7 (p = 0.0026). Another multipoint linkage peak was observed on chromosome 16q21-22 (83 cM; LOD=1.5, p = 0.0040). Prioritization analysis revealed Cellular tumor antigen p53 (TP53, MIM# 191170), a candidate gene for schizophrenia, as the strongest gene related to schizophrenia in the 17p region (p=0.0006) (Table S2). Nevertheless, none of the prioritized genes located under the multipoint linkage peaks for openness fulfilled our criterion (Table S2), and were therefore not examined for differential expression in the brain of schizophrenia patients versus controls.

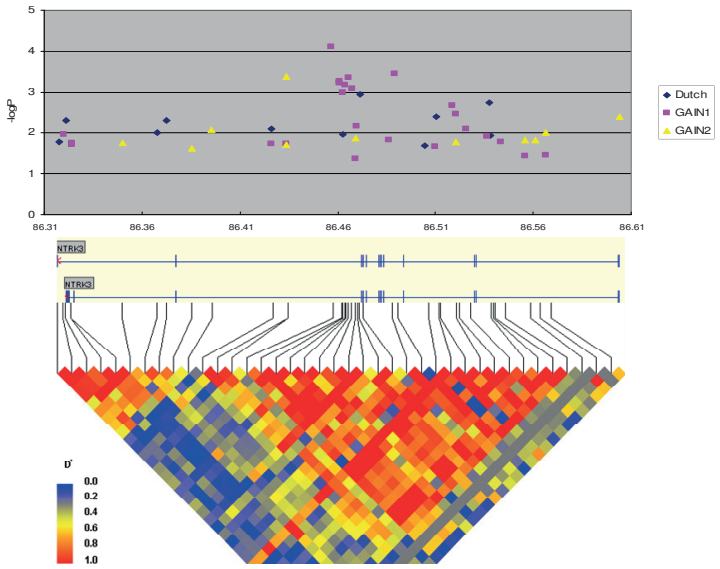
Verbal Fluency A two-point linkage peak with a borderline-significant empirical p-value of 0.00002 and LOD-score of 2.5 was obtained for verbal fluency at 9q31-33 (rs10081701), close to two other suggestive two-point linkage peaks for verbal fluency (Table 2). In multipoint linkage analysis this region showed a LOD-score of 0.9. We identified 6 genes of interest near SNPs with suggestive evidence for linkage with verbal fluency, based on prioritization (Table S2). Three of these genes showed a differential expression in brain tissue of patients with schizophrenia compared to control subjects in published datasets (Supplementary Tables S2 and S3) and fulfilled our criterion for pursuing association analysis: EIF4E2 (Eukaryotic translation initiation factor 4E type 2; MIM#605895) and GIGYF2 (PERQ amino acid-rich with GYF domain-containing protein 2, MIM#612003) within the vicinity of SNP rs7286, and RGS3 (Regulator of G-protein signaling 3, MIM#602189) within the vicinity of rs10081701. Subsequent association analysis showed no associations between SNPs within GIGYF2 or RGS2 and schizophrenia (data not shown), while EIF4E2 showed associations with schizophrenia for several SNPs, though only one SNP remained significant after correction by permutation (Tables 4 and S4).

Early visual perception Two-point linkage analysis yielded a LOD of 1.7 (empirical p = 0.001) for early visual perception at 20p13 for SNP rs12624577. The highest multipoint linkage peak was located at 8q24.2-24.3 (LOD=1.8, p = 0.0022; Supplementary Figure S6). Other multipoint peaks of interest were located at 2p21 (LOD=1.5), 5q35.1-35.3 (LOD=1.34), 21q21.3 (LOD=1.24), and Xp22.2 (LOD=1.39). In prioritization analysis of genes in the two regions with a LOD higher than 1.5, the PTK2 (Focal adhesion kinase, MIM#1600758) gene mapped on 8q24.2 was prioritized as the most relevant candidate gene for schizophrenia based on its functional characteristics (Table S2). However, PTK2 was not differentially expressed in the brain in schizophrenia versus controls and therefore not included in the subsequent association analysis.

Spatial working memory Two-point linkage revealed several suggestive peaks for spatial working memory, i.e. at 15q26.1 for SNP rs8025499 (LOD=1.68, empirical p =0.0004), at Xp22.11 for SNP rs739974 (LOD=1.98, empirical p =0.0002), and at Xq21.31-22.1 for SNPs rs761843

and rs2030392 (LOD=1.92 and 1.70; empirical p=0.0002 and 0.0004, respectively). Multipoint analysis resulted in decreased LOD scores for these regions. When these genomic regions were prioritized, we found the NTRK3 (neurotrophic tyrosine kinase, receptor, type 3, MIM#191316) gene within the vicinity of rs8025499 at 15q26.1 to be functionally related to other candidate genes for schizophrenia (p=0.001). In two independent studies, this gene showed a differential expression (Tables S2 and S3). NTRK3 showed a decreased expression in the prefrontal cortex of schizophrenia patients when compared to control subjects (p= 0.039),[45] whereas patients had increased expression levels in the cerebellum compared to control subjects (p= 0.009; GEO accession GDS1917) (Table S3). When testing for association, 19 of the 100 SNPs in NTRK3 showed nominal association with schizophrenia (at unadjusted p-value < 0.05) mostly in a dominant model (Table S4). Twelve SNPs remained significant after correcting for the number of SNPs in the four genes tested through 10,000 permutations (Table S4). The rs1105962 SNP had a significantly (p<0.002) lower frequency of the "G" allele (0.500) in patients with schizophrenia compared to control subjects (0.582). When we performed a haplotype analysis using a window of 5 SNPs moving across the 19 SNPs covering the entire NTRK3 and associated linkage disequilibrium (LD) blocks (Supplementary Table S5), one particular haplotype with a sequence of "GAAGG" reached a significance level of p=0.0006 for an association with schizophrenia (haplotype rs10520672 - rs9806762, F_a: 0.201 vs. F_u: 0.254). We subsequently tested the NTRK3

Figure 1 –Log P values and linkage disequilibrium (D') for SNPs in NTRK3 that were significantly associated (p<0.05) with schizophrenia in one of the three association samples



Note: The x-axis of the plot represents position in Mb (Build 36). Only 2 of the 6 transcripts of NTRK3 are shown, which were all similar for the part shown.

locus for association in two additional (independent) schizophrenia case-control samples of European and Afro-American descent (GAIN data, see Web resources; $N_{case/control}$= 1,172/1,378 and 921/954). Again, several SNPs (in high LD with some of the identified SNPs in the Dutch sample; Figure 1) showed an associated signal with schizophrenia (49 of the 147 SNPs at $p<0.05$; Supplementary Table S6). SNP A-2298768 had a significantly ($p<0.00008$) higher frequency of the "G" allele (0.451) in patients with schizophrenia compared to control subjects (0.382). These findings further strengthened the case for involvement of NTRK3 in the susceptibility for schizophrenia and related cognitive endophenotypes.

IQ Intelligence was suggestively linked to a region at 2q22.1 in two-point analysis (SNP rs1918615, LOD=1.78, empirical p=0.00013), which also generated the highest multipoint LOD for IQ of 0.9. A potential candidate gene underneath this peak may be the LRP1B (Low-density lipoprotein receptor-related protein 1B precursor, MIM608766) gene, as it is more likely to be related to pathways relating to schizophrenia candidate genes than expected (p=0.001). However, it did not fulfill our criterion for further testing.

Discussion

We performed a genome-wide scan for quantitative-trait loci (QTLs) that influence variation in cognitive endophenotypes for schizophrenia in multigenerational multiply affected families from the Netherlands. The endophenotypes included were previously shown to be heritable[11] and to some extent genetically correlated.[24] Although we did not detect significant linkage, three regions of interest emerged from the multipoint analysis: 8q21-24 for early visual perception, and 16q21-22 and 17p13 for openness. Additionally, suggestive two-point peaks were obtained on several locations that overlapped or closely bordered to previous linkage findings for schizophrenia, e.g., at chromosome 5q23.2-34, 9q31-33, and 15q26.1, or related endophenotypes, such as event-related potentials during a working memory task at chromosome 9q31-33, and a composite neurocognitive phenotype and IQ at chromosome 2q22.1. We examined the suggestive findings for relevant genes and observed accumulative evidence in support of NTRK3 as a susceptibility gene for schizophrenia and candidate endophenotype spatial working memory.

Multipoint linkage Our most prominent multipoint linkage finding is a peak (LOD 1.8) on 8q24 for early visual perception. Although this region has not been linked to schizophrenia previously, it has been linked to P50/antisaccade phenotype,[19] IQ,[22] and bipolar disorder (MIM125480) in several independent studies.[4,46] Bipolar disorder is genetically closely related to schizophrenia,[47] and has been associated with deficits in early visual perception.[48] Our finding for this region

supports the hypothesis that genes in this region may be related to psychosis and related phenotypes.

Two other multipoint linkage findings of interest were obtained for openness at 16q21-22 and 17p13.2-13.1. These regions overlap with or are situated close (<10cM) to previous significant[49] and suggestive[50,51] linkage peaks for schizophrenia, and associated SNPs in recent genome-wide association (GWA) studies for bipolar disorder[52,53] and schizophrenia.[54] Also, linkage peaks for joint schizotypy and schizophrenia,[55] and suggestive linkage findings for a variety of endophenotypes[20,23,56,57] have been observed in these regions. Interestingly, both regions were highlighted for Attention-Deficit Hyperactivity Disorder (ADHD; MIM143456) in a recent meta-analysis,[58] suggesting a correlation between openness-to-experience, which can be characterized as cognitive flexibility, exploration, curiousness or being unconventional, and ADHD. However, several studies did not detect such a correlation.[59] Our data supports the idea that these regions may be involved in neurocognition and psychiatric disorders. Our prioritization analysis suggested several genes of interest that are mapped to this region, e.g. the candidate gene for schizophrenia TP53.

Two-point linkage Verbal fluency yielded most of the suggestive two-point peaks. The highest peak for verbal fluency, at 9q31-33, is located close to linkage findings for schizophrenia,[60,61] event-related potentials during a working memory task,[62] and schizotypy,[55] and harbors a gene of interest with regard to schizophrenia, RGS3. However, none of the SNPs within this gene were associated with schizophrenia in the Dutch sample.

Notably, there is overlap between meta-analysis loci (ranked) for schizophrenia and several of our candidate loci. A locus at 5q23.2-34 for sensorimotor gating is located within a region that ranked 2nd and 1st in meta-analyses of linkage studies in schizophrenia[5,6] and contains a cluster of GABA(A) receptor genes, among which is one of the strongest candidate genes for schizophrenia, GABRB2 (SZgene website[43] see Web resources). These GABA(A) receptors may have contributed to our LOD-score for sensorimotor gating, as suggested by animal research.[63] A two-point peak of interest for IQ was observed on 2q22.1 and lies within the 5th ranked locus for schizophrenia in a recent meta-analysis,[6] near a SNP at 2q22.2 that was associated to schizophrenia at a genome-wide significance level,[64] and adjacent (~6-22cM) to several peaks for neurocognitive phenotypes and IQ.[14,21,23] Although power was low for IQ in this study, the overlap with multiple previous findings for IQ and schizophrenia supports the possibility of a yet unknown QTL at 2q22 with strong effects on cognition and schizophrenia. For spatial working memory we obtained suggestive linkage peaks at 15q26.1 and Xp22.11 that lie respectively near and within meta-analysis loci for schizophrenia.[4,5] Even though not significantly, two-point link-

age revealed several loci of interest, as shown by simulation, overlap with previous linkage regions for schizophrenia and related traits and the positional candidate genes residing here.

Schizophrenia candidate genes Assuming that the endophenotypes we studied are genetically related to schizophrenia, we examined our endophenotype linkage findings by searching for genes underneath the peaks that are functionally inter-related and related to schizophrenia. This was done through performing prioritization analyses, examining expression, and performing association analyses in a Dutch case-control sample, all with regard to schizophrenia. Accumulative evidence highlighted NTRK3 as a gene of interest for schizophrenia. Subsequently, we replicated our result in two independent schizophrenia case-control samples by observing yet stronger associations between NTRK3 and schizophrenia in LD with our previous findings.

The NTRK3 gene, located at the 15q26 region linked to spatial working memory, encodes a member of the neurotrophic tyrosine receptor kinase family and is involved in nervous system development and myelination.[65] The biochemical pathways of NTRK3, including neurotrophic receptors, have previously been implicated in schizophrenia.[66,67] NTRK3 exerts effects on neuregulin (NRG1)[68] and interacts with neurotrophin 3 (NTF3),[65] both of which have been associated with schizophrenia.[43,69] NTRK3 may act on spatial working memory through dopaminergic neurons[70] in combination with its effects on the dorsolateral prefrontal cortex (DLPFC), where the expression of NTRK3 is significantly reduced.[71] Our finding for a relation between NTRK3 and schizophrenia is further emphasized as markers in NTRK3 have been significantly associated with several psychiatric disorders, including mood disorder (MIM608516),[72] eating disorder,[73] and obsessive-compulsive hoarding (MIM164130),[74] and recently, with schizophrenia.[75] Interestingly, one SNP in NTRK3 which is in strong LD with our most significant SNPs, has been linked with hippocampal activity.[75] In summary, our findings together with those of functional, and epidemiological studies consistently highlight NTRK3 as a candidate gene for schizophrenia and spatial working memory.

Regardless of the interesting findings we recognize some limitations in our study, especially the limited statistical power due to a relatively small sample size and the necessity for multiple testing. This may bias the results towards no signal particularly in multipoint linkage analysis, which depends on distributional characteristics of the traits within families. Nevertheless, most of the two-point linkage findings are of interest, as they were supported by simulation, by overlap with previously identified genomic regions for schizophrenia and related endophenotypes, and by the identification of several suggestive candidate genes for schizophrenia. We reported the two-point linkage findings which passed the genome-wide simulation in linkage analysis or those which passed the 10,000 permutation significance in association analysis. Concerning the association

between NTRK3 and schizophrenia, the replication of our finding in separate samples ($p=8*10^{-5}$) suggests this gene to be involved in the vulnerability to schizophrenia.

Our identification of NTRK3 as candidate susceptibility gene was based on the assumption of a genetic correlation between endophenotypes and schizophrenia. This may be considered another limitation of our study; however, multiple studies have shown deficits of these endophenotypes in both schizophrenia patients and their relatives.[11] Conservatively, we may have missed genes by using prioritization analyses that are biased for known biological pathways,[41] including a small region of 1 Mb surrounding the two-point markers in prioritization, and examining expression of genes only in the prefrontal and cerebellar tissues of the brain.

Our study illustrates some of the complexity of incorporating endophenotypes in genetic research, including heterogeneity of the traits. Nevertheless, we have been able to combine various levels of scientific information, characterizing a full-range strategy, augmenting the depth of studying endophenotypes and schizophrenia, which is desired when dealing with complex phenotypes and when genetic effects are likely to be small.[76]

Summarizing, our results, together with prior studies, indicate several potential QTLs for early visual perception, openness, sensorimotor gating, verbal fluency, spatial working memory, and IQ, where susceptibility genes for schizophrenia reside, i.e., GABRB2, TP53, NTRK3. Our suggestive QTLs require replication in larger samples, and if sustaining, warrant further study, such as fine-mapping of the candidate region, detection of disequilibrium, and, ultimately, identification of one or more functional polymorphisms. Subsequently, based on prioritization of positional candidate genes, expression, and association in three independent samples, our findings show accumulative evidence of the involvement of NTRK3 in a cognitive endophenotype for schizophrenia. Further studies are necessary to confirm this finding and to reveal the NTRK3 regulation and function in the brain and potential predisposition to schizophrenia.

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Web Resources

http://www.ncbi.nlm.nih.gov/Omim/
http://pngu.mgh.harvard.edu/purcell/plink/
http://www.schizophreniaforum.org/res/sczgene
http://www.ncbi.nlm.nih.gov/sites/GDSbrowser?acc=GDS1917/
http://www.ncbi.nlm.nih.gov/gap

Online Supplementary Material

Supplementary Table S1 Training set of candidate genes used in Endeavour prioritization analysis

DISC1, SLC18A1, GRIN2B, DRD2, PLXNA2, AKT1, DGCR2, RPGRIP1L, TPH1, DRD4, DAOA, DRD1, HTR2A, RELN, APOE, NRG1, IL1B, MTHFR, HP, DAO, ZNF804A, DTNBP1, OPCML, RGS4, (GABRB2, TP53).

Supplementary Table S2 Results of prioritization analyses and expression data.

Linkage				Prioritization							Expression a		
Trait	Chromosome	Marker	Position [b]	Gene	Symbol	Description	Prioritizer Network # c	Prioritizer p	Endeavour p	Trait(s) d	CCHPC	HBB	GDS1917
SG	5	rs728693	156724866	ENSG00000113263	ITK	Tyrosine-protein kinase ITK/TSK	-	0.820	0.0377	SG_SWM			
				ENSG00000135074	ADAM19	ADAM 19 precursor	-	0.862	0.00314	SG_SWM			
				ENSG00000155858	LSM11	U7 snRNA-associated Sm-like protein LSm11	7	0.859	0.385	SG_SWM			
	5	rs13178296	161064178	ENSG00000145864	GABRB2	Gamma-aminobutyric-acid receptor subunit beta-2 precursor	-	0.873	0.0435	SG_SWM			
				ENSG00000022355	GABRA1	Gamma-aminobutyric-acid receptor subunit alpha-1 precursor	-	0.881	0.00266	SG_SWM			
				ENSG00000113327	GABRG2	Gamma-aminobutyric-acid receptor subunit gamma-2 precursor	-	0.833	0.0128	SG_SWM			
OP	16	16q21-22.1	64110065	ENSG00000166592	RRAD	GTP-binding protein RAD	-	0.583	0.0145	OP			
				ENSG00000172828	CES3	esterase 31	6	0.548	0.429	OP			
				ENSG00000172824	NP_776176.2		6	0.384	-	OP			
				ENSG00000102871	TRADD	Tumor necrosis factor receptor type 1-associated DEATH domain protein	-	0.831	0.0176	OP			
				ENSG00000159720	ATP6V0D1	Vacuolar ATP synthase subunit d	-	0.521	0.0295	OP			
	17	17p13.2-13.1	5742055	ENSG00000141503	MINK1	Misshapen-like kinase 1	-	0.277	0.00426	OP			
				ENSG00000108556	CHRNE	Acetylcholine receptor subunit epsilon precursor	-	0.500	0.0152	OP			
				ENSG00000185245	GP1BA	Platelet glycoprotein Ib alpha chain precursor	-	0.990	0.0108	OP			
				ENSG00000129250	KIF1C	Kinesin-like protein KIF1C	-	0.954	0.028	OP			
				ENSG00000029725	RABEP1	Rab GTPase-binding effector protein 1	-	0.994	0.011	OP			
				ENSG00000108561	C1QBP	Complement component 1 Q subcomponent-binding protein, mitochondrial precursor	-	1	0.0149	OP			
				ENSG00000091592	NLRP1	NACHT, LRR and PYD-containing protein 1	-	0.702	0.0166	OP			
				ENSG00000141505	ASGR1	Asialoglycoprotein receptor 1	-	0.977	0.0444	OP			
				ENSG00000132535	DLG4	Discs large homolog 4	-	1	0.00207	OP			
				ENSG00000072778	ACADVL	Very-long-chain specific acyl-CoA dehydrogenase, mitochondrial precursor	-	0.507	0.026	OP			
				ENSG00000132522	GPS2	G protein pathway suppressor 2	-	0.948	0.0272	OP			
				ENSG00000072818	CENTB1|KCTD11	Centaurin-beta 1	6	0.317	0.0845	OP			
				ENSG00000169992	NLGN2	Neuroligin-2 precursor	-	1	0.00246	OP			
				ENSG00000161958	FGF11	Fibroblast growth factor 11	-	0.980	0.0232	OP			
				ENSG00000170175	CHRNB1	Acetylcholine receptor subunit beta precursor	-	0.253	0.00313	OP			
				ENSG00000129214	SHBG	Sex hormone-binding globulin precursor	-	0.989	0.0151	OP			
				ENSG00000129244	ATP1B2	Sodium/potassium-transporting ATPase subunit beta-2	-	0.992	0.0179	OP			
				ENSG00000141510	TP53	Cellular tumor antigen p53	-	0.984	0.000557	OP			
				ENSG00000108947	EFNB3	Ephrin-B3 precursor	-	0.984	0.0196	OP			
VF	1	rs1194580	152486109	ENSG00000143614	GATAD2B	Transcriptional repressor p66 beta	13	0.130	0.132	VF			
				ENSG00000143614	GATAD2B	Transcriptional repressor p66 beta	18	0.230	0.134	VF_IQ			
				ENSG00000143578	CREB3L4	cAMP responsive element binding protein 3-like 4	13	0.118	0.33	VF			
				ENSG00000143578	CREB3L4	cAMP responsive element binding protein 3-like 4	18	0.215	0.334	VF_IQ			
				ENSG00000143543	JTB	Protein JTB precursor	14	0.103	0.0539	VF			
				ENSG00000143543	JTB	Protein JTB precursor	19	0.202	0.0561	VF_IQ			
				ENSG00000177954	RPS27	40S ribosomal protein S27	11	0.114	0.256	VF			
				ENSG00000177954	RPS27	40S ribosomal protein S27	16	0.217	0.269	VF_IQ			
				ENSG00000143575	HAX1	HS1-associating protein X-1	14	0.122	0.518	VF			
				ENSG00000143575	HAX1	HS1-associating protein X-1	19	0.223	0.531	VF_IQ			
				ENSG00000160712	IL6R	Interleukin-6 receptor alpha chain precursor	-	0.206	0.0446	VF			
				ENSG00000160712	IL6R	Interleukin-6 receptor alpha chain precursor	-	0.336	0.0471	VF_IQ			

Supplementary Table S2 (continued)

Trait	Chromosome	Marker	Position [b]	Gene	Symbol	Description	Prioritizer Network # c	Prioritizer p	Endeavour p	Trait(s) d	CCHPC	HBB	GDS1917
				ENSG00000160716	CHRNB2	Neuronal acetylcholine receptor subunit beta-2 precursor	-	0.133	0.00364	VF			
				ENSG00000160716	CHRNB2	Neuronal acetylcholine receptor subunit beta-2 precursor	-	0.243	0.00406	VF_IQ			
				ENSG00000160710	**ADAR**	Double-stranded RNA-specific adenosine deaminase	11	0.116	0.0488	VF	-	-	-
				ENSG00000160710	**ADAR**	Double-stranded RNA-specific adenosine deaminase	16	0.223	0.0526	VF_IQ	-	-	-
	1	rs2066981	153439003	ENSG00000160691	**SHC1**	SHC-transforming protein 1	12	0.080	0.00531	VF	-	-	-
				ENSG00000160691	**SHC1**	SHC-transforming protein 1	20	0.184	0.00542	VF_IQ	-	-	-
				ENSG00000143537	ADAM15	ADAM 15 precursor	-	0.128	0.00329	VF			
				ENSG00000143537	ADAM15	ADAM 15 precursor	-	0.240	0.00398	VF_IQ			
				ENSG00000185499	MUC1	Mucin-1 precursor	-	1	0.0439	VF_IQ			
				ENSG00000185499	MUC1	Mucin-1 precursor	-	1	0.0446	VF			
				ENSG00000176444	CLK2	Dual specificity protein kinase CLK2	-	0.138	0.0317	VF			
				ENSG00000176444	CLK2	Dual specificity protein kinase CLK2	-	0.253	0.032	VF_IQ			
				ENSG00000160752	FDPS	Farnesyl pyrophosphate synthetase	-	0.139	0.0227	VF			
				ENSG00000160752	FDPS	Farnesyl pyrophosphate synthetase	-	0.257	0.0234	VF_IQ			
				ENSG00000116539	ASH1L	Probable histone-lysine N-methyltransferase ASH1L	-	0.378	0.0483	VF			
				ENSG00000132676	DAP3	Mitochondrial 28S ribosomal protein S29	14	0.093	0.0995	VF			
				ENSG00000132676	DAP3	Mitochondrial 28S ribosomal protein S29	19	0.198	0.109	VF_IQ			
	2	rs7286	233255229	ENSG00000163283	ALPP|ALPPL2	Alkaline phosphatase, placental type precursor	15	0.101	0.128	VF			
				ENSG00000163283	ALPP|ALPPL2	Alkaline phosphatase, placental type precursor	17	0.215	0.133	VF_IQ			
				ENSG00000163286	ALPPL2	Alkaline phosphatase, placental-like precursor	15	0.0228	0.312	VF			
				ENSG00000163286	ALPPL2	Alkaline phosphatase, placental-like precursor	17	0.0252	0.332	VF_IQ			
				ENSG00000163295	ALPI	Intestinal alkaline phosphatase precursor	15	0.095	0.178	VF			
				ENSG00000163295	ALPI	Intestinal alkaline phosphatase precursor	17	0.206	0.194	VF_IQ			
				ENSG00000171551	ECEL1	Endothelin-converting enzyme-like 1	-	0.550	0.0307	VF			
				ENSG00000171551	ECEL1	Endothelin-converting enzyme-like 1	-	0.574	0.0321	VF_IQ			
				ENSG00000135902	CHRND	Acetylcholine receptor subunit delta precursor	-	0.100	0.000162	VF			
				ENSG00000135902	CHRND	Acetylcholine receptor subunit delta precursor	-	0.207	0.000178	VF_IQ			
				ENSG00000135930	**EIF4E2**	Eukaryotic translation initiation factor 4E type 2	11	0.122	0.03	VF	-	-	**0.022 (3)**
				ENSG00000135930	**EIF4E2**	Eukaryotic translation initiation factor 4E type 2	16	0.234	0.0319	VF_IQ	-	-	**0.022 (3)**
				ENSG00000168918	INPP5D		12	0.110		VF			
				ENSG00000168918	INPP5D		20	0.219		VF_IQ			
				ENSG00000204120	**GIGYF2**	PERQ amino acid-rich with GYF domain-containing protein 2	-	-	0.00722	VF_IQ	-	-	**0.012 (5)**
				ENSG00000204120	**GIGYF2**	PERQ amino acid-rich with GYF domain-containing protein 2	-	-	0.00723	VF	-	-	**0.012 (5)**
	2	rs838715	233957423	ENSG00000077044	DGKD	Diacylglycerol kinase delta	12	0.209	0.108	VF			
				ENSG00000077044	DGKD	Diacylglycerol kinase delta	20	0.353	0.113	VF_IQ			
				ENSG00000167165	UGT1A1	UDP-glucuronosyltransferase 1-8 precursor	-	0.147	0.0292	VF			
				ENSG00000167165	UGT1A1	UDP-glucuronosyltransferase 1-8 precursor	-	0.265	0.0299	VF_IQ			
	9	rs10081701	115011250	ENSG00000138835	**RGS3**	Regulator of G-protein signaling 3	-	-	0.00787	VF	**0.0112**	-	-
				ENSG00000138835	**RGS3**	Regulator of G-protein signaling 3	-	-	0.00815	VF_IQ	**0.0112**	-	-
	9	rs1516882	107952732	ENSG00000186051	TAL2	T-cell acute lymphocytic leukemia-2 protein	13	0.150	0.588	VF			
				ENSG00000186051	TAL2	T-cell acute lymphocytic leukemia-2 protein	18	0.289	0.602	VF_IQ			
	10	rs1892302	12486578	ENSG00000151461	**UPF2**	Regulator of nonsense transcripts 2	11	0.095	0.0109	VF	-	-	-
				ENSG00000151461	**UPF2**	Regulator of nonsense transcripts 2	16	0.201	0.0118	VF_IQ	-	-	-

Supplementary Table S2 (continued)

Trait	Chromosome	Marker	Position [b]	Gene	Symbol	Description	Prioritizer Network # [c]	Prioritizer p	Endeavour p	Trait(s) [d]	CCHPC	HBB	GDS1917
				ENSG00000181192	DHTKD1	dehydrogenase E1 and transketolase domain containing protein 1	-	0.0400	0.128	VF			
				ENSG00000165609	NUDT5	ADP-sugar pyrophosphatase	15	0.055	0.591	VF			
				ENSG00000165609	NUDT5	ADP-sugar pyrophosphatase	17	0.147	0.614	VF_IQ			
	10	rs4615920	13382660	ENSG00000165630	PRPF18	Pre-mRNA-splicing factor 18	11	0.216	0.326	VF			
				ENSG00000165630	PRPF18	Pre-mRNA-splicing factor 18	16	0.359	0.337	VF_IQ			
EVP	2	2p21	46365480	ENSG00000068784	SRBD1	S1 RNA binding domain 1	5	0.075	0.296				
				ENSG00000171132	PRKCE	Protein kinase C epsilon type	-	0.483	0.0092	EVP			
				ENSG00000171132	PRKCE	Protein kinase C epsilon type	-	0.897	0.00333	EVP_SWM			
				ENSG00000116016	EPAS1	Endothelial PAS domain-containing protein 1	-	0.879	0.00375	EVP_SWM			
				ENSG00000116016	EPAS1	Endothelial PAS domain-containing protein 1	-	0.952	0.0144	EVP			
				ENSG00000171142	ATP6V1E2	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E isoform 2	2	0.873	0.78	EVP_SWM			
				ENSG00000119729	RHOQ	Rho-related GTP-binding protein RhoQ	-	0.114	0.0333	EVP			
				ENSG00000119729	RHOQ	Rho-related GTP-binding protein RhoQ	-	0.880	0.0103	EVP_SWM			
				ENSG00000119878	CRIPT	postsynaptic protein CRIPT	-	0.880	0.0307	EVP_SWM			
				ENSG00000171142	CALM2	Calmodulin	-	-	0.0168	EVP_SWM			
				ENSG00000143933	CALM2	Calmodulin	-	-	0.0313	EVP			
	8	8q24.2-24.3	139363006	ENSG00000104472	CHRAC1	Chromatin accessibility complex protein 1	1	0.865	0.27	EVP_SWM			
				ENSG00000169398	**PTK2**	Focal adhesion kinase 1	-	0.437	0.0283	EVP	-	-	-
				ENSG00000169398	**PTK2**	Focal adhesion kinase 1	3	0.851	0.0116	EVP_SWM	-	-	-
SWM	15	rs8025499	86990988	ENSG00000140538	**NTRK3**	NT-3 growth factor receptor precursor	-	0.837	0.00262	SG_SWM	0.0392	-	**0.009 (5)**
				ENSG00000140538	**NTRK3**	NT-3 growth factor receptor precursor	3	0.882	0.00165	EVP_SWM	0.0392	-	**0.009 (5)**
				ENSG00000140538	**NTRK3**	NT-3 growth factor receptor precursor	-	0.908	0.00132	SWM	0.0392	-	**0.009 (5)**
				ENSG00000140538	**NTRK3**	NT-3 growth factor receptor precursor	-	0.946	0.00165	SWM_IQ	0.0392	-	**0.009 (5)**
				ENSG00000181991	MRPS11	28S ribosomal protein S11, mitochondrial precursor	8	0.781	0.376	SG_SWM			
				ENSG00000181991	MRPS11	28S ribosomal protein S11, mitochondrial precursor	9	0.828	0.306	SWM			
				ENSG00000181991	MRPS11	28S ribosomal protein S11, mitochondrial precursor	4	0.840	0.307	EVP_SWM			
				ENSG00000181991	MRPS11	28S ribosomal protein S11, mitochondrial precursor	10	0.898	0.374	SWM_IQ			
				ENSG00000172183	ISG20	Interferon-stimulated gene 20 kDa protein	5	0.772	0.219	EVP_SWM			
				ENSG00000157766	ACAN	Aggrecan core protein precursor	-	0.861	0.000777	SG_SWM			
				ENSG00000157766	ACAN	Aggrecan core protein precursor	-	0.883	0.00108	EVP_SWM			
				ENSG00000157766	ACAN	Aggrecan core protein precursor	-	0.900	0.000618	SWM			
				ENSG00000157766	ACAN	Aggrecan core protein precursor	-	0.943	0.000826	SWM_IQ			
				ENSG00000140526	ABHD2	Abhydrolase domain-containing protein 2	-	0.0480	0.325	EVP_SWM			
	X	rs2030392	92449164	ENSG00000186310	NAP1L3	Nucleosome assembly protein 1-like 3	1	0.731	0.17	EVP_SWM			
	X	rs761843	100203746	ENSG00000000003	TSPAN6	Tetraspanin-6	-	0.0164	0.0552	SWM_IQ			
				ENSG00000000003	TSPAN6	Tetraspanin-6	-	0.0228	0.0507	SWM			
				ENSG00000000003	TSPAN6	Tetraspanin-6	-	0.0264	0.0683	SG_SWM			
				ENSG00000000003	TSPAN6	Tetraspanin-6	-	0.0272	0.0561	EVP_SWM			
				ENSG00000101811	CSTF2	Cleavage stimulation factor 64 kDa subunit	7	0.860	0.448	SG_SWM			
				ENSG00000101811	CSTF2	Cleavage stimulation factor 64 kDa subunit	5	0.872	0.494	EVP_SWM			
				ENSG00000007952	NOX1	NADPH oxidase homolog 1	2	0.577	0.169	EVP_SWM			

Supplementary Table S2 (continued)

Trait	Chromosome	Marker	Position [b]	Gene	Symbol	Description	Prioritizer Network # [c]	Prioritizer p	Endeavour p	Trait(s) [d]	CCHPC	HBB	GDS1917
				ENSG00000010671	BTK	Tyrosine-protein kinase BTK	-	0.842	0.0104	SG_SWM			
				ENSG00000010671	BTK	Tyrosine-protein kinase BTK	-	0.846	0.00529	EVP_SWM			
				ENSG00000010671	BTK	Tyrosine-protein kinase BTK	-	0.892	0.00534	SWM			
				ENSG00000010671	BTK	Tyrosine-protein kinase BTK	-	0.942	0.00613	SWM_IQ			
				ENSG00000102391	RPL36A	60S ribosomal protein L36a	8	0.846	0.56	SG_SWM			
				ENSG00000102391	RPL36A	60S ribosomal protein L36a	4	0.853	0.502	EVP_SWM			
				ENSG00000102391	RPL36A	60S ribosomal protein L36a	9	0.876	0.521	SWM			
				ENSG00000102391	RPL36A	60S ribosomal protein L36a	10	0.930	0.551	SWM_IQ			
				ENSG00000126945	HNRPH2	Heterogeneous nuclear ribonucleoprotein H\\\'	7	0.860	0.112	SG_SWM			
				ENSG00000126945	HNRPH2	Heterogeneous nuclear ribonucleoprotein H\\\'	5	0.870	0.167	EVP_SWM			
IQ	2	rs1918615	140514213	ENSG00000168702	LRP1B	Low-density lipoprotein receptor-related protein 1B precursor	-	0.392	0.00128	VF_IQ			
				ENSG00000168702	LRP1B	Low-density lipoprotein receptor-related protein 1B precursor	-	0.930	0.00564	SWM_IQ			

Note: only genes that were prioritized in one of the two methods (using threshold of p =0.05 or being part of small network (Prioritizer) are provided in the table. Bolded genes were selected for each region to be reviewed for expression in published and online datasets, based on the criterion of: a) being significantly prioritized with a p<0.01 in Endeavour or <0.05 in Prioritizer, or b) being part of a direct network in Prioritizer and yielding a p<0.05 in Endeavour. If neither criterion was met for any gene in the region we selected the gene with the best p-value (if <0.05) or being part of a small network in Prioritizer. Bolded expression p-values indicate the expression of the gene is increased in schizophrenia (normal fond indicates decreased expression). P-values were not corrected for multiple testing. Abbreviations: CCHPC: Charing Cross Hospital prospective collection (N Cases/controls: 28/23, [53]; GDS1917: GEO accession GDS1917, see Web resources; HBB: Harvard Brain Bank (N Cases/controls: 16/27; [52,53]; SG: sensorimotor gating; OP: openness; VF: verbal fluency; EVP: early visual perception; SWM: spatial working memory; IQ: intelligence; snw: small network in Prioritizer, SCZ: schizophrenia.

[a] If empty than expression was not reviewed; a dash indicates that genes were either not available or not significant; in between brackets the number of probes tested; for mean intensity values and test statistics please refer to [53] for HBB and CCHPC samples and online material for the GDS1917-study (see Web Resources).

[b] Positions in bp, Build 36.

[c] Numbers are arbitrary and indicate the networks of genes observed by Prioritizer. A dash indicates non-significance (P>0.05).

[d] The trait or combination of traits for which regions were analysed in prioritization analysis. We did not perform analyses on the single regions for SG and IQ separately.

Supplementary Table S3 Results of Mann-Whitney U test on expression levels of prioritized genes in cerebellar cortex tissue of schizophrenia patients versus control subjects (GEO accession GDS1917, see Web resources)

Prioritized Gene	Probe	Median intensity controls	N	Median intensity schizophrenia	N	U	Exact Significance [a]	Effect size
ADAR	201786_s_ADAR	1570.9	14	1557.9	14	89	0.701	-0.078
EIF4E2	p226734_at_EIF4E2	278.098	14	260.606	14	70	0.21	-0.243
	p209393_s_at_EIF4E2	93.6885	7	89.0948	6	5	**0.022**	-0.634
	p213571_s_at_EIF4E2	69.2621	14	80.4857	14	63	0.114	-0.304
GIGYF2	p212261_at_GIGYF2	362.94	14	326.012	14	81	0.454	-0.148
	p212260_at_GIGYF2	170.695	14	162.186	12	53	0.118	-0.313
	p1560133_at_GIGYF2	104.234	14	97.8614	14	88	0.667	-0.087
	p237052_x_at_GIGYF2	81.4484	14	72.675	14	67	0.164	-0.269
	p1558305_at_GIGYF2	29.6512	9	41.2708	5	4	**0.012**	-0.659
NTRK3	p206462_s_at_NTRK3	119.178	14	166.789	14	42	**0.009**	-0.486
	p215025_at_NTRK3	75.164	10	83.0594	10	34	0.247	-0.270
	p215115_x_at_NTRK3	129.063	14	142.11	14	60	0.085	-0.330
	p217033_x_at_NTRK3	124.199	14	145.694	14	72	0.246	-0.226
	p228849_at_NTRK3	190.457	14	206.455	14	69	0.194	-0.252
PTK2	207821_s_PTK2	436.0	14	400.6	14	93	0.839	-0.043
	1559529_PTK2	101.0	14	99.6	14	87	0.635	-0.096
	208820_PTK2	854.0	14	855.9	14	93	0.839	-0.043
RGS3	203823_RGS3	114.1	10	106.3	10	35	0.28	-0.254
SHC1	not testable: 'Detection call' = Absent							
UPF2	203519_s_UPF2	528.8	14	516.9	14	88	0.667	-0.087

[a] 2*(1-tailed Significance).

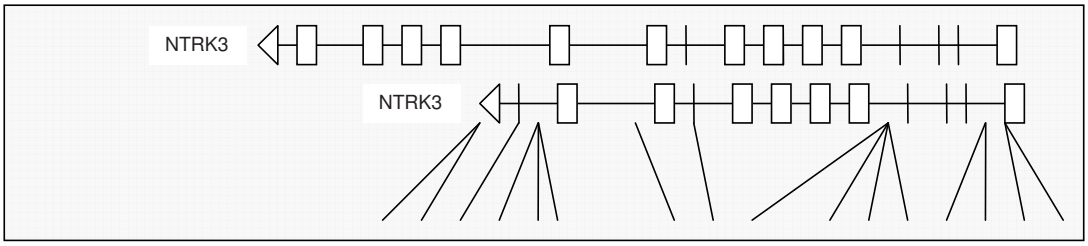
Supplementary Table S4 Results of association analysis comparing 758 schizophrenia cases with 676 control subjects from the Netherlands on four genes of interest based on prioritization and expression

				Schizophrenia patients		Control subjects		Quality control				
Chr	Gene name	SNP	Position	Genotype counts (AA/AB/BB)	MAF	Genotype counts (AA/AB/BB)	MAF	Missing rate	P HWE in controls	Best Model	P best model	Empirical significance threshold
2	EIF4E2	rs1550097	233136519	46/244/465	0.223	36/265/374	0.250	0.003	0.258	Dominant	0.0178	0.0171
2	EIF4E2	rs1190456	233143348	47/235/474	0.218	35/261/380	0.245	0.001	0.298	Genotypic	0.0110	0.0105
2	EIF4E2	rs6749955	233146161	45/249/464	0.224	34/264/374	0.247	0.003	0.177	Dominant	**0.0332**	0.0336
15	NTRK3	rs7172184	86317392	147/395/216	0.455	166/315/193	0.480	0.001	0.105	Recessive	0.0167	0.0157
15	NTRK3	rs1461213	86321295	119/395/243	0.418	107/302/264	0.383	0.003	0.192	Dominant	0.00492	0.00460
15	NTRK3	rs11073757	86324401	167/386/197	0.480	155/285/195	0.469	0.034	0.013	Genotypic	0.0461	0.0438
15	NTRK3	rs1841551	86342645	79/343/334	0.331	71/267/336	0.303	0.003	0.102	Dominant	**0.0319**	0.0363
15	NTRK3	rs10520672	86367621	70/335/348	0.315	64/253/358	0.282	0.004	0.057	Dominant	0.0101	0.00990
15	NTRK3	rs4887348	86372538	62/309/380	0.288	49/233/390	0.246	0.008	0.096	Dominant	**0.00495**	0.00510
15	NTRK3	rs7176834	86425418	1/0/752	0.001	1/6/665	0.006	0.006	0.021	Allelic	**0.0372**	0.0937
15	NTRK3	rs17755717	86426100	28/224/500	0.186	17/166/489	0.149	0.007	0.541	Allelic	**0.00784**	0.00810
15	NTRK3	rs9806762	86462743	92/362/299	0.363	84/365/223	0.397	0.006	0.001	Dominant	0.0107	0.0100
15	NTRK3	rs16941321	86471762	62/310/381	0.288	59/332/283	0.334	0.005	0.006	Dominant	**0.00113**	0.00190
15	NTRK3	rs12148845	86504550	38/261/454	0.224	33/274/363	0.254	0.008	0.042	Dominant	**0.0199**	0.0230
15	NTRK3	rs12594095	86508197	38/254/461	0.219	26/270/378	0.239	0.005	0.008	Genotypic	0.0370	0.0369
15	NTRK3	rs10520676	86510642	31/242/480	0.202	27/267/378	0.239	0.006	0.019	Dominant	**0.00391**	0.00480
15	NTRK3	rs16941364	86513598	35/250/470	0.212	29/264/380	0.239	0.004	0.056	Dominant	**0.0261**	0.0289
15	NTRK3	rs8025158	86514696	35/251/468	0.213	32/263/380	0.242	0.003	0.141	Dominant	**0.0266**	0.0277
15	NTRK3	rs1105962	86537313	65/312/377	0.293	59/334/282	0.335	0.003	0.004	Dominant	**0.00185**	0.00270
15	NTRK3	rs4887381	86538332	36/246/472	0.211	27/269/377	0.240	0.005	0.015	Dominant	**0.0115**	0.0127
15	NTRK3	rs4887212	86557205	112/380/264	0.400	130/332/212	0.439	0.003	1.000	Recessive	0.0243	0.0238
15	NTRK3	rs1863488	86574283	12/135/610	0.105	5/150/519	0.119	0.002	0.138	Genotypic	0.0448	0.0223

Note: Only SNPs with a nominal p<0.05 are shown. P-values in bold are below the empirical significance threshold correcting for the number of SNPs within the four genes tested through 10,000 permutations.

Abbreviations: Chr: chromosome; MAF: minor allele frequency.

Supplementary Table S5 Allelotype and haplotype-based association of NTRK3 in a Dutch sample of schizophrenia patients (n= 758) versus control subjects (n= 676)



						snp	rs7172184	rs1461213	rs11073757	rs1841551	rs10520672	rs4887348	rs7176834	rs17755717	rs9806762	rs16941321	rs12148845	rs12594095	rs10520676	rs16941364	rs8025158	rs1105962	rs4887381	rs4887212	rs1863488
						model	rec	dom	geno	dom	dom	dom	Allelic	Allelic	dom	dom	dom	geno	dom	dom	dom	dom	dom	rec	geno
F_a	F_u	CHISQ	DF	p		p	**0.0167**	**0.0049**	**0.0461**	**0.0319***	**0.0101**	**0.0049***	**0.0372***	**0.0078***	**0.0107**	**0.0011***	**0.0199***	**0.0370**	**0.0039***	**0.0261***	**0.0266***	**0.0019***	**0.0115***	**0.0243**	**0.0448**
0.020	0.010	4.71	1	**0.03006**			A	G	A	G	A														
0.020	0.010	4.75	1	**0.02925**				G	A	G	A	G													
0.022	0.010	5.40	1	**0.02017**					A	G	A	G	A												
0.072	0.052	4.65	1	**0.03110**						G	A	G	A	A											
0.055	0.037	5.21	1	**0.02250**							A	G	A	A	A										
0.054	0.037	4.50	1	**0.03394**								G	A	A	A	A									
0.132	0.103	5.35	1	**0.02076**									A	A	A	A	A								
0.133	0.113	2.39	1	0.12180										A	A	A	A	A							
0.626	0.596	2.56	1	0.10950											A	A	A	A	G						
0.702	0.663	4.92	1	**0.02656**												A	A	A	G	A					
0.757	0.737	1.55	1	0.21260													A	A	G	A	G				
0.665	0.632	3.38	1	0.06609														A	G	A	G	A			
0.686	0.642	6.06	1	**0.01383**															G	A	G	A	C		
0.476	0.435	4.58	1	**0.03244**																A	G	A	C	G	
0.478	0.440	4.07	1	**0.04367**																	G	A	C	G	G
0.016	0.027	4.137	1	**0.04196**			G	G	G	G	G														
0.016	0.027	4.415	1	**0.03563**				G	G	G	G	A													
0.017	0.028	4.127	1	**0.04220**					G	G	G	A	A												
0.017	0.028	4.073	1	**0.04357**						G	G	A	A	G											
0.569	0.618	6.823	1	**0.00900**						A	G	A	A	G											
0.2	0.254	11.91	1	**0.00056**							G	A	A	G	G										
0.177	0.223	9.513	1	**0.00204**								A	A	G	G	C									
0.057	0.076	3.927	1	**0.04750**									A	G	G	C	A								
0.054	0.076	5.897	1	**0.01516**										G	G	C	A	A							
0.056	0.077	4.924	1	**0.02648**											G	C	A	A	G						
0.054	0.075	4.9	1	**0.02686**												C	A	A	G	A					
0.757	0.737	1.554	1	0.21260													A	A	G	A	G				
0.096	0.107	1.033	1	0.30940														A	G	A	G	G			
0.057	0.071	2.28	1	0.13110															G	A	G	G	C		
0.057	0.08	6.226	1	**0.01259**																A	G	G	C	A	
0.055	0.077	5.33	1	**0.02096**																	G	G	C	A	G

Abbreviations: F_a and F_u: frequency in affected and unaffected individuals, respectively. Note: Horizontal p-values are based on model-based tests, vertical p-values on haplotype-based tests. P-values below 0.05 are bolded.

* P-values are below empirical significance threshold.

Supplementary Table S6 Results of Association between NTRK3 and schizophrenia in a Dutch sample and two replication (GAIN) samples of European and Afro-American descent

		Dutch					GAIN1					GAIN2				
SNP	Position	MAF SCZ	MAF C	Model	P	EST	MAF SCZ	MAF C	Model	P	EST	MAF SCZ	MAF C	Model	P	EST
SNP_A-8550291	86233175											0.342	0.375	Dominant	**0.0302**	0.0315
SNP_A-1894085	86258643						0.397	0.381	Recessive	**0.0258**	0.0265					
SNP_A-2303067	86263503						0.168	0.147	Dominant	**0.0407**	0.0685					
rs7172184	86317392	0.455	0.480	Recessive	**0.0167**	0.0184										
SNP_A-8707979	86320220						0.338	0.372	Trend	0.0109	0.0103					
rs1461213	86321295	0.418	0.383	Dominant	**0.00492**	0.0053										
SNP_A-1804855	86324120						0.500	0.474	Recessive	**0.0191**	0.0198					
SNP_A-8703195	86324325						0.500	0.475	Recessive	**0.0179**	0.0195					
rs11073757	86324401	0.480	0.469	Genotypic	0.0461	0.0452										
rs1841551	86342645	0.331	0.303	Dominant	0.0319	0.0301										
SNP_A-2282959	86350155											0.325	0.341	Recessive	0.0177	0.0176
rs10520672	86367621	0.315	0.282	Dominant	0.0101	0.0095										
rs4887348	86372538	0.288	0.246	Dominant	**0.00495**	0.0051										
SNP_A-1803546	86385314											0.107	0.127	Dominant	**0.0241**	0.0250
SNP_A-8600718	86395443											0.177	0.193	Recessive	**0.00849**	0.0125
rs7176834	86425418	0.001	0.006	Allelic	**0.0372**	0.0972										
SNP_A-2303210	86426100						0.184	0.159	Dominant	**0.0182**	0.0372					
rs17755717	”	0.186	0.149	Allelic	0.00784	0.0069										
SNP_A-2029994	86433890						0.415	0.447	Trend	**0.0187**	0.0191	0.153	0.175	Recessive	0.000418	0.0003
SNP_A-2299374	86433931											0.481	0.514	Recessive	**0.0190**	0.0190
SNP_A-1787536	86436684											0.190	0.218	Dominant	**0.0346**	0.0350
SNP_A-2304183	86452753						0.414	0.445	Trend	**0.0228**	0.0259					
SNP_A-8318255	86454854						0.411	0.441	Trend	**0.0288**	0.0292					
SNP_A-8500731	86455529						0.411	0.440	Trend	**0.0295**	0.0312					
SNP_A-2298768	86456524						0.262	0.214	Dominant	**0.0000797**	0.0003					
SNP_A-8488020	86460910						0.253	0.213	Trend	**0.000549**	0.0006					
SNP_A-8599956	86461119						0.277	0.235	Trend	**0.000591**	0.0007					

Supplementary Table S6 (continued)

		Dutch					GAIN1					GAIN2				
SNP	Position	MAF SCZ	MAF C	Model	P	EST	MAF SCZ	MAF C	Model	P	EST	MAF SCZ	MAF C	Model	P	EST
SNP_A-8372616	86462636						0.255	0.216	Trend	0.00104	0.0010					
rs9806762	86462743	0.363	0.397	Dominant	0.0107	0.0093										
SNP_A-8710246	86463950						0.253	0.213	Trend	**0.000676**	0.0007					
SNP_A-8622812	86465680						0.277	0.235	Trend	0.000442	0.0003					
SNP_A-1824765	86467650						0.274	0.234	Trend	**0.000821**	0.0009					
SNP_A-8586229	86468952						0.397	0.424	Recessive	**0.0427**	0.0480	0.190	0.222	Trend	0.0131	0.0125
SNP_A-8697071	86469695						0.223	0.195	Recessive	**0.00687**	0.0077					
rs16941321	86471762	0.288	0.334	Dominant	**0.00113**	0.0012										
SNP_A-2267884	86486334						0.453	0.419	Dominant	**0.0148**	0.0289					
SNP_A-2196872	86489014						0.227	0.187	Trend	**0.000351**	0.0006					
SNP_A-2197678	86494637											0.467	0.432	Dominant	**0.0278**	0.0279
rs12148845	86504550	0.224	0.254	Dominant	**0.0199**	0.0201										
rs12594095	86508197	0.219	0.239	Genotypic	**0.0370**	0.0392										
SNP_A-2296946	86510061						0.058	0.072	Dominant	0.0220	0.0134					
rs10520676	86510642	0.202	0.239	Dominant	0.00391	0.0036										
rs16941364	86513598	0.212	0.239	Dominant	0.0261	0.0250										
rs8025158	86514696	0.213	0.242	Dominant	0.0266	0.0250										
SNP_A-2303312	86515555											0.299	0.289	Genotypic	0.0442	0.0423
SNP_A-2270058	86518712						0.251	0.215	Trend	**0.00215**	0.0023					
SNP_A-8463733	86520227						0.473	0.431	Dominant	**0.00337**	0.0200	0.175	0.205	Allelic	**0.0167**	0.0170
SNP_A-2075600	86525651						0.244	0.213	Trend	0.00789	0.0073					
SNP_A-8711023	86536314						0.228	0.200	Trend	**0.0118**	0.0126					
rs1105962	86537313	0.293	0.335	Dominant	**0.00185**	0.0020										
rs4887381	86538332	0.211	0.240	Dominant	0.0115	0.0110										
SNP_A-4244712	86543443											0.211	0.191	Genotypic	0.0194	0.0193
SNP_A-8697472	86543516						0.085	0.067	Trend	0.0162	0.0160					
SNP_A-2210736	86544398											0.216	0.190	Dominant	0.0259	0.0256
SNP_A-8651992	86554721						0.134	0.115	Dominant	**0.0415**	0.0734					
SNP_A-4204914	86555933						0.135	0.116	Dominant	**0.0353**	0.0594					

Supplementary Table S6 (continued)

		Dutch					GAIN1					GAIN2				
SNP	Position	MAF SCZ	MAF C	Model	P	EST	MAF SCZ	MAF C	Model	P	EST	MAF SCZ	MAF C	Model	P	EST
SNP_A-8597980	86556014						0.134	0.114	Dominant	**0.0372**	0.0678	0.382	0.356	Dominant	**0.0149**	0.0168
SNP_A-1795683	86556308						0.132	0.114	Dominant	**0.0482**	0.0776					
SNP_A-8423443	86557205						0.433	0.405	Dominant	**0.0408**	0.0637					
rs4887212	”	0.400	0.439	Recessive	**0.0243**	0.0245										
SNP_A-4303567	86560842											0.470	0.430	Allelic	**0.0148**	0.0151
SNP_A-2303365	86561203						0.133	0.113	Dominant	**0.0359**	0.0554					
SNP_A-8452734	86562798						0.132	0.114	Dominant	**0.0480**	0.0617					
SNP_A-2173892	86564157						0.133	0.113	Dominant	**0.0318**	0.0447					
SNP_A-2274000	86566368						0.137	0.117	Dominant	**0.0344**	0.0554	0.471	0.429	Allelic	**0.00981**	0.0102
SNP_A-8610296	86571378											0.203	0.194	Recessive	0.0372	0.0322
rs1863488	86574283	0.105	0.119	Genotypic	0.0448	0.0216										

Note: only SNPs with a p-value<0.05 are shown. SNPs in bold are below the empirically derived significance thresholds. In total 209 SNPs were tested: 99 in the Dutch sample and 109 in the GAIN samples.

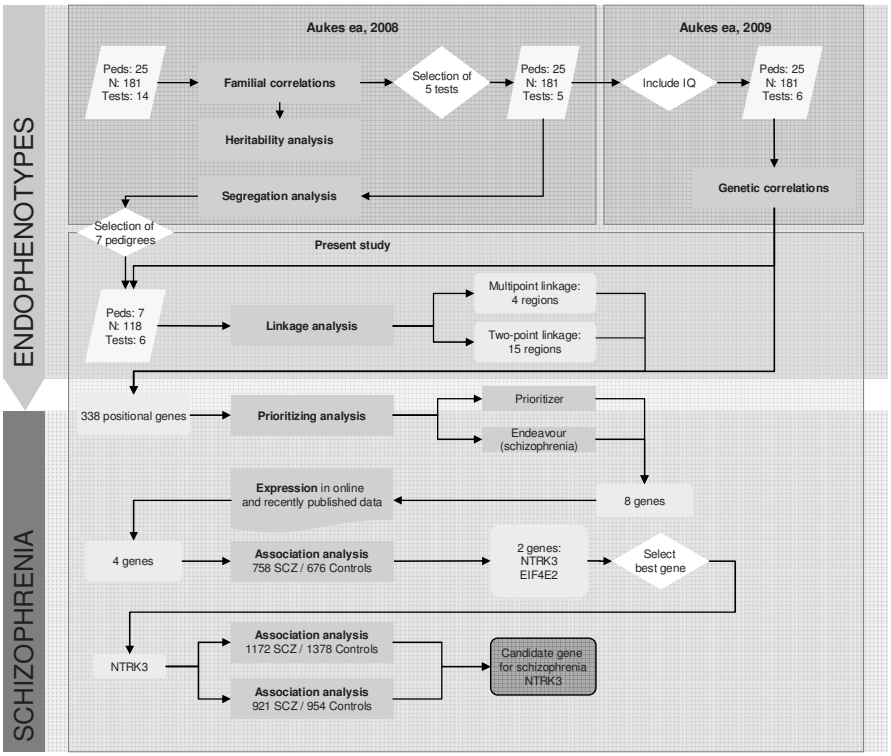
Abbreviations: MAF: minor allele frequency; SCZ/C: schizophrenia/controls; P: p-value best model; EST: Empirical significance threshold.

[a] The Dutch sample consisted of 758 schizophrenia patients and 676 control subjects.

[b] The GAIN1 sample consisting of 1,172 schizophrenia patients and 1,378 control subjects.

[c] The GAIN2 sample consisted of 921 schizophrenia patients and 954 control subject.

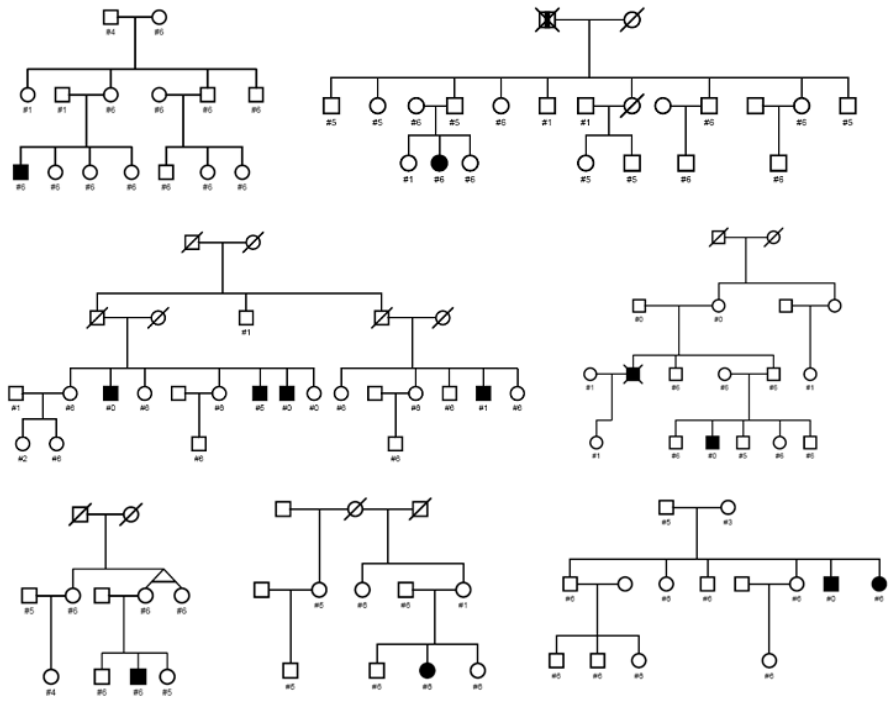
Supplementary Figure S1 Study design depicting previous selection analyses and present analyses



Note: Diagonals indicate dataset, samples and phenotypes; rectangles (sharp corners) indicate statistical analyses; diamonds indicate decisions; rectangles (rounded corners) indicate results.

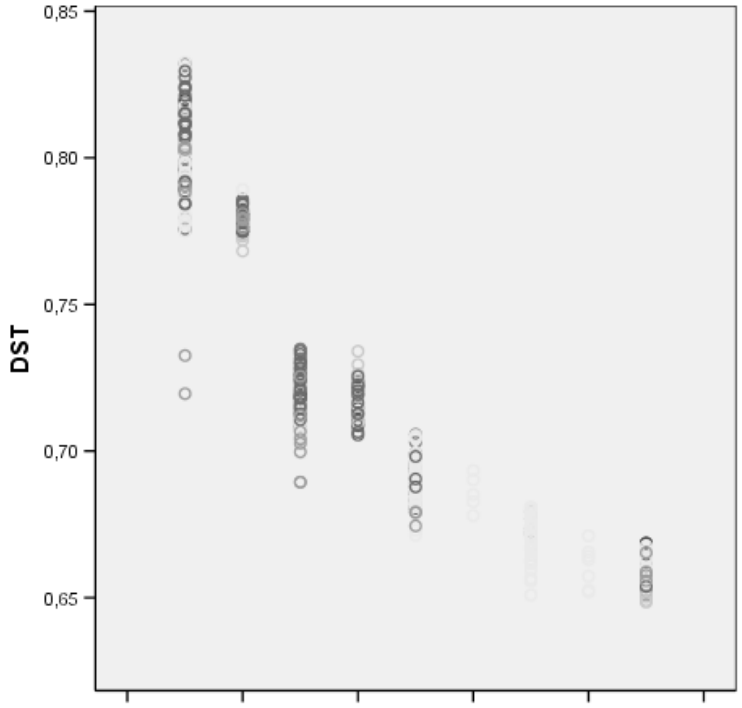
Abbreviations: Peds: pedigrees; N: number of individuals; SCZ: schizophrenia.

Supplementary Figure S2 Linkage pedigrees



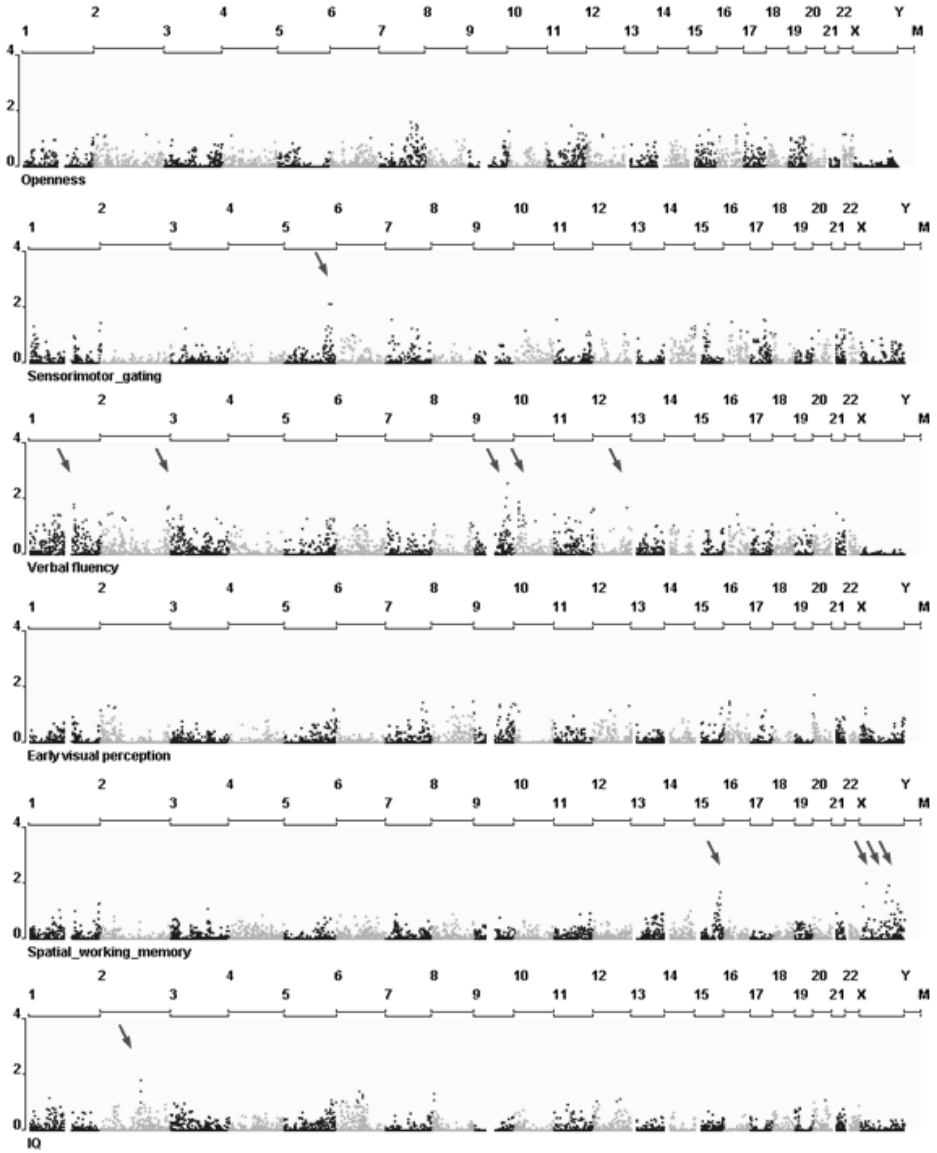
Note: in order to disguise the pedigrees sex and birth order have been changed randomly. Individuals that were genotyped are marked with the number of endophenotypes tested.

Supplementary Figure S3 Identity by state (IBS) distance plotted against the expected familial relationships. Two sib-pairs in a sibship of 3 individuals clearly deviate compared to relatedness of other sib-pairs



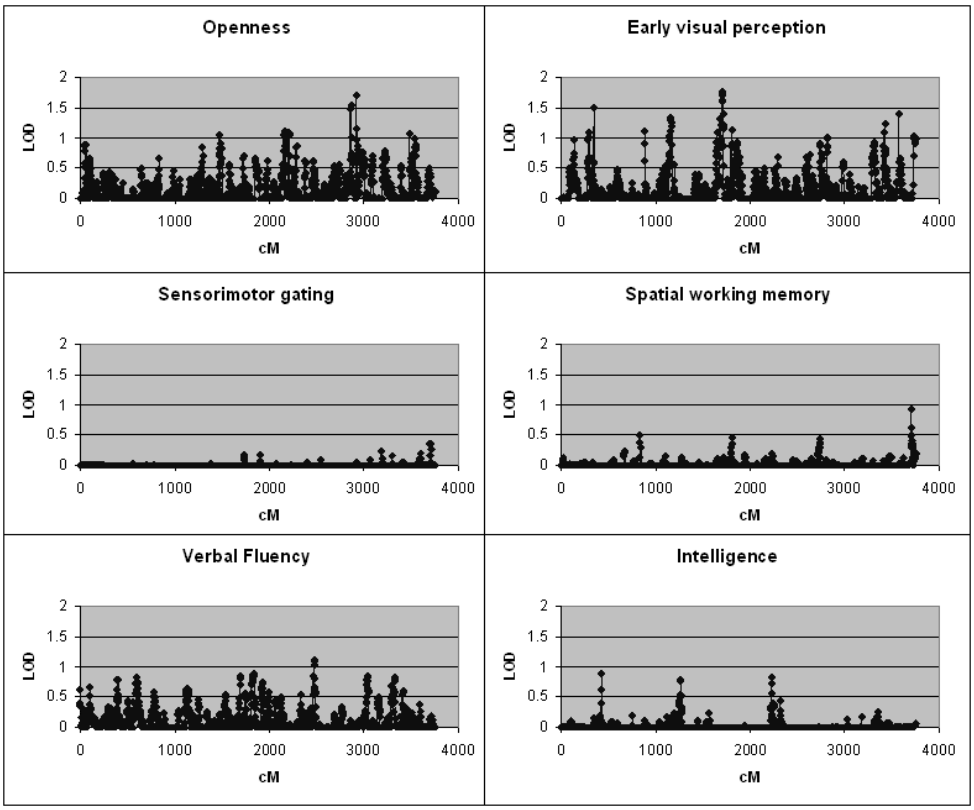
Note: X-axis represents kinship, from left to right: siblings (1), parent-offspring (2), Aunt/Uncle-Niece/nephew (3), grandparent-grandchild (4), cousins (5), grandaunt/uncle (6), first cousins once removed (7), second cousins (8), unrelated (9). DST: distance = (IBS2 + 0.5*IBS1)/N SNP pairs.

Supplementary Figure S4 Genome-wide two-point linkage results for six endophenotypes



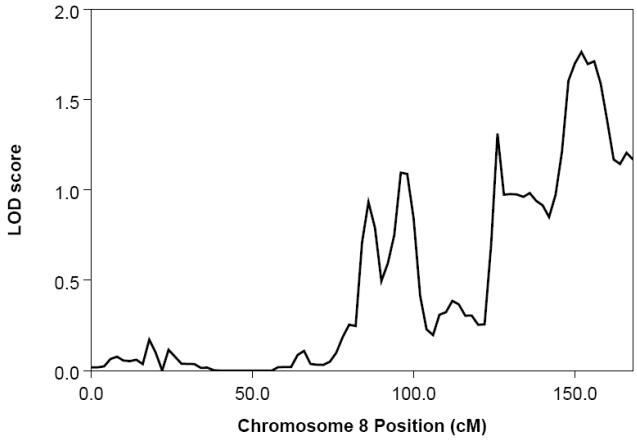
Note: The x-axis represents the consecutive chromosomes with numbers indicated above the figures; the y-axis represents LOD scores. Arrows indicate suggestive two-point findings.

Supplementary Figure S5 Genome-wide multipoint results for all traits



Note: Positions on the x-axis are in cumulative cM (DeCode) over all 22 autosomes and the X chromosome

Supplementary Figure S6 Multipoint linkage peak for early visual perception on chromosome 8



References

1. McGrath J, Saha S, Chant D, Welham J (2008): Schizophrenia: a concise overview of incidence, prevalence, and mortality. *Epidemiol Rev* 30: 67-76.
2. Sullivan PF, Kendler KS, Neale MC (2003): Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* 60: 1187-1192.
3. McGue M, Gottesman II, Rao DC (1985): Resolving genetic models for the transmission of schizophrenia. *Genet Epidemiol* 2: 99-110.
4. Badner JA, Gershon ES (2002): Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 7: 405-411.
5. Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I et al (2003): Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 73: 34-48.
6. Ng MY, Levinson DF, Faraone SV, Suarez BK, DeLisi LE, Arinami T et al (2008): Meta-analysis of 32 genome-wide linkage studies of schizophrenia. *Mol Psychiatry* 14: 774-85.
7. Gottesman II, Gould TD (2003): The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 160: 636-645.
8. Erlenmeyer-Kimling L, Rock D, Roberts SA, Janal M, Kestenbaum C, Cornblatt B et al (2000): Attention, memory, and motor skills as childhood predictors of schizophrenia-related psychoses: the New York High-Risk Project. *Am J Psychiatry* 157: 1416-1422.
9. Heinrichs RW, Zakzanis KK (1998): Neurocognitive deficit in schizophrenia: a quantitative review of the evidence. *Neuropsychology* 12: 426-445.
10. Sitskoorn MM, Aleman A, Ebisch SJ, Appels MC, Kahn RS (2004): Cognitive deficits in relatives of patients with schizophrenia: a meta-analysis. *Schizophr Res* 71: 285-295.
11. Aukes MF, Alizadeh BZ, Sitskoorn MM, Selten JP, Sinke RJ, Kemner C et al (2008): Finding Suitable Phenotypes for Genetic Studies of Schizophrenia: Heritability and Segregation Analysis. *Biol Psychiatry* 64: 128-36.
12. Gasperoni TL, Ekelund J, Huttunen M, Palmer CG, Tuulio-Henriksson A, Lonnqvist J et al (2003): Genetic linkage and association between chromosome 1q and working memory function in schizophrenia. *Am J Med Genet* 116B: 8-16.
13. Paunio T, Tuulio-Henriksson A, Hiekkalinna T, Perola M, Varilo T, Partonen T et al (2004): Search for cognitive trait components of schizophrenia reveals a locus for verbal learning and memory on 4q and for visual working memory on 2q. *Hum Mol Genet* 13: 1693-1702.
14. Hallmayer JF, Kalaydjieva L, Badcock J, Dragovic M, Howell S, Michie PT et al (2005): Genetic evidence for a distinct subtype of schizophrenia characterized by pervasive cognitive deficit. *Am J Hum Genet* 77: 468-476.
15. Almasy L, Gur RC, Haack K, Cole SA, Calkins ME, Peralta JM et al (2008): A genome screen for quantitative trait loci influencing schizophrenia and neurocognitive phenotypes. *Am J Psychiatry* 165: 1185-1192.
16. Arolt V, Lencer R, Nolte A, Muller-Myhsok B, Purmann S, Schurmann M et al (1996): Eye tracking dysfunction is a putative phenotypic susceptibility marker of schizophrenia and maps to a locus on chromosome 6p in families with multiple occurrence of the disease. *Am J Med Genet* 67: 564-579.
17. Freedman R, Coon H, Myles-Worsley M, Orr-Urtreger A, Olincy A, Davis A et al (1997): Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proc Natl Acad Sci U S A* 94: 587-592.
18. Matthysse S, Holzman PS, Gusella JF, Levy DL, Harte CB, Jorgensen A et al (2004): Linkage of eye movement dysfunction to chromosome 6p in schizophrenia: additional evidence. *Am J Med Genet B Neuropsychiatr Genet* 128: 30-36.
19. Myles-Worsley M, Coon H, McDowell J, Brenner C, Hoff M, Lind B et al (1999): Linkage of a composite inhibitory phenotype to a chromosome 22q locus in eight Utah families. *Am J Med Genet* 88: 544-550.
20. Takahashi S, Ohtsuki T, Yu SY, Tanabe E, Yara K, Kamioka M et al (2003): Significant linkage to chromosome 22q for exploratory eye movement dysfunction in schizophrenia. *Am J Med Genet B Neuropsychiatr Genet* 123: 27-32.

21. Posthuma D, Luciano M, Geus EJ, Wright MJ, Slagboom PE, Montgomery GW et al (2005): A genomewide scan for intelligence identifies quantitative trait loci on 2q and 6p. *Am J Hum Genet* 77: 318-326.
22. Dick DM, Aliev F, Bierut L, Goate A, Rice J, Hinrichs A et al (2006): Linkage analyses of IQ in the collaborative study on the genetics of alcoholism (COGA) sample. *Behav Genet* 36: 77-86.
23. Luciano M, Wright MJ, Duffy DL, Wainwright MA, Zhu G, Evans DM et al (2006): Genome-wide scan of IQ finds significant linkage to a quantitative trait locus on 2q. *Behav Genet* 36: 45-55.
24. Aukes MF, Alizadeh BZ, Sitskoorn MM, Kemner C, Ophoff RA, Kahn RS (2009): Genetic overlap among intelligence and other candidate endophenotypes for schizophrenia. *Biol Psychiatry* 65: 527-534.
25. Ott J (1989): Computer-simulation methods in human linkage analysis. *Proc Natl Acad Sci U S A* 86: 4175-4178.
26. National Institute of Mental Health (1991): *Molecular Genetics Initiative: Family Interview for Genetic Studies.* Bethesda, MD, National Institute of Mental Health.
27. Andreasen NC, Flaum M, Arndt S (1992): The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnosis and psychopathology. *Arch Gen Psychiatry* 49: 615-623.
28. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E et al (1998): The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 59 Suppl 20: 22-33.
29. Costa PT Jr., McCrae RR (1992): *Revised NEO Personality Inventory (NEO-PI-R) and NEO Five-Factor Inventory (NEO-FFI) professional manual.* Odessa, FL: Psychological Assessment Resources, Inc.
30. Luteijn F, Van der Ploeg F (1983): *The Groninger Intelligence Test (in Dutch).* Lisse, the Netherlands, Swets & Zeitlinger B.V.
31. Green MF, Nuechterlein KH, Breitmeyer B (2002): Development of a computerized assessment for visual masking. *Int J Methods Psychiatr Res* 11: 83-89.
32. Wechsler D (1997): *Wechsler Memory Scale (3rd ed.).* San Antonio, TX, The Psychological Corporation.
33. Wechsler D (2000): *WAIS-III Nederlandstalige Bewerking. Afname en scoringshandleiding.* Lisse, Netherlands, Swets Test Publishers.
34. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D et al (2007): PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81: 559-575.
35. O'connell JR, Weeks DE (1998): PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 63: 259-266.
36. Abecasis GR, Cherny SS, Cookson WO, Cardon LR (2002): Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 30: 97-101.
37. Bartels I, Schlueter G, Liehr T, von EF, Starke H, Glaubitz R et al (2003): Supernumerary small marker chromosome (SMC) and uniparental disomy 22 in a child with confined placental mosaicism of trisomy 22: trisomy rescue due to marker chromosome formation. *Cytogenet Genome Res* 101: 103-105.
38. Wigginton JE, Abecasis GR (2005): PEDSTATS: descriptive statistics, graphics and quality assessment for gene mapping data. *Bioinformatics* 21: 3445-3447.
39. Allison DB, Neale MC, Zannolli R, Schork NJ, Amos CI, Blangero J (1999): Testing the robustness of the likelihood-ratio test in a variance-component quantitative-trait loci-mapping procedure. *Am J Hum Genet* 65: 531-544.
40. Lander E, Kruglyak L (1995): Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11: 241-247.
41. Franke L, van BH, Fokkens L, de Jong ED, Egmont-Petersen M, Wijmenga C (2006): Reconstruction of a functional human gene network, with an application for prioritizing positional candidate genes. *Am J Hum Genet* 78: 1011-1025.
42. Aerts S, Lambrechts D, Maity S, Van LP, Coessens B, De SF et al (2006): Gene prioritization through genomic data fusion. *Nat Biotechnol* 24: 537-544.

43. Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ et al (2008): Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat Genet* 40: 827-834.
44. Glatt SJ, Everall IP, Kremen WS, Corbeil J, Sasik R, Khanlou N et al (2005): Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia. *Proc Natl Acad Sci U S A* 102: 15533-15538.
45. Maycox PR, Kelly F, Taylor A, Bates S, Reid J, Logendra R et al (2009): Analysis of gene expression in two large schizophrenia cohorts identifies multiple changes associated with nerve terminal function. *Mol Psychiatry* March 2009, advance online publication, doi: 10.1038/mp.2009.18.
46. Segurado R, tera-Wadleigh SD, Levinson DF, Lewis CM, Gill M, Nurnberger JI, Jr. et al (2003): Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: Bipolar disorder. *Am J Hum Genet* 73: 49-62.
47. Lin PI, Mitchell BD (2008): Approaches for unraveling the joint genetic determinants of schizophrenia and bipolar disorder. *Schizophr Bull* 34: 791-797.
48. Green MF, Nuechterlein KH, Mintz J (1994): Backward masking in schizophrenia and mania. I. Specifying a mechanism. *Arch Gen Psychiatry* 51: 939-944.
49. Bulayeva KB, Glatt SJ, Bulayev OA, Pavlova TA, Tsuang MT (2007): Genome-wide linkage scan of schizophrenia: a cross-isolate study. *Genomics* 89: 167-177.
50. Faraone SV, Matise T, Svrakic D, Pepple J, Malaspina D, Suarez B et al (1998): Genome scan of European-American schizophrenia pedigrees: results of the NIMH Genetics Initiative and Millennium Consortium. *Am J Med Genet* 81: 290-295.
51. DeLisi LE, Shaw SH, Crow TJ, Shields G, Smith AB, Larach VW et al (2002): A genome-wide scan for linkage to chromosomal regions in 382 sibling pairs with schizophrenia or schizoaffective disorder. *Am J Psychiatry* 159: 803-812.
52. Wellcome Trust Case Control Consortium (2007): Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447: 661-678.
53. Sklar P, Smoller JW, Fan J, Ferreira MA, Perlis RH, Chambert K et al (2008): Whole-genome association study of bipolar disorder. *Mol Psychiatry* 13: 558-569.
54. Sullivan PF, Lin D, Tzeng JY, van den OE, Perkins D, Stroup TS et al (2008): Genomewide association for schizophrenia in the CATIE study: results of stage 1. *Mol Psychiatry* 13: 570-584.
55. Fanous AH, Neale MC, Gardner CO, Webb BT, Straub RE, O'Neill FA et al (2007): Significant correlation in linkage signals from genome-wide scans of schizophrenia and schizotypy. *Mol Psychiatry* 12: 958-965.
56. Nash MW, Huezo-Diaz P, Williamson RJ, Sterne A, Purcell S, Hoda F et al (2004): Genome-wide linkage analysis of a composite index of neuroticism and mood-related scales in extreme selected sibships. *Hum Mol Genet* 13: 2173-2182.
57. Francks C, Fisher SE, MacPhie IL, Richardson AJ, Marlow AJ, Stein JF et al (2002): A genomewide linkage screen for relative hand skill in sibling pairs. *Am J Hum Genet* 70: 800-805.
58. Zhou K, Dempfle A, rcos-Burgos M, Bakker SC, Banaschewski T, Biederman J et al (2008): Meta-analysis of genome-wide linkage scans of attention deficit hyperactivity disorder. *Am J Med Genet B Neuropsychiatr Genet* 147B: 1392-1398.
59. Miller CJ, Miller SR, Newcorn JH, Halperin JM (2008): Personality characteristics associated with persistent ADHD in late adolescence. *J Abnorm Child Psychol* 36: 165-173.
60. Kaufmann CA, Suarez B, Malaspina D, Pepple J, Svrakic D, Markel PD et al (1998): NIMH Genetics Initiative Millenium Schizophrenia Consortium: linkage analysis of African-American pedigrees. *Am J Med Genet* 81: 282-289.
61. Fallin MD, Lasseter VK, Wolyniec PS, McGrath JA, Nestadt G, Valle D et al (2003): Genomewide linkage scan for schizophrenia susceptibility loci among Ashkenazi Jewish families shows evidence of linkage on chromosome 10q22. *Am J Hum Genet* 73: 601-611.
62. Hansell NK, Medland SE, Ferreira MA, Geffen GM, Zhu G, Montgomery GW et al (2006): Linkage analyses of event-related potential slow wave phenotypes recorded in a working memory task. *Behav Genet* 36: 29-44.
63. Takahashi K, Nagai T, Kamei H, Maeda K, Matsuya T, Arai S et al (2007): Neural circuits containing pallidotegmental GABAergic neurons are involved in the prepulse inhibition of the startle reflex in mice. *Biol Psychiatry* 62: 148-157.

64. O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V et al (2008): Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet* 40: 1053-1055.
65. Cosgaya JM, Chan JR, Shooter EM (2002): The neurotrophin receptor p75NTR as a positive modulator of myelination. *Science* 298: 1245-1248.
66. Davis KL, Haroutunian V (2003): Global expression-profiling studies and oligodendrocyte dysfunction in schizophrenia and bipolar disorder. *Lancet* 362: 758.
67. Durany N, Thome J (2004): Neurotrophic factors and the pathophysiology of schizophrenic psychoses. *Eur Psychiatry* 19: 326-337.
68. Hippenmeyer S, Shneider NA, Birchmeier C, Burden SJ, Jessell TM, Arber S (2002): A role for neuregulin1 signaling in muscle spindle differentiation. *Neuron* 36: 1035-1049.
69. Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S et al (2002): Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet* 71: 877-892.
70. von Bohlen und Halbach O, Minichiello L, Unsicker K (2005): Haploin-sufficiency for trkB and trkC receptors induces cell loss and accumulation of {alpha}-synuclein in the substantia nigra. *FASEB J* 19: 1740-1742.
71. Weickert CS, Ligons DL, Romanczyk T, Ungaro G, Hyde TM, Herman MM et al (2005): Reductions in neurotrophin receptor mRNAs in the prefrontal cortex of patients with schizophrenia. *Mol Psychiatry* 10: 637-650.
72. Feng Y, Vetro A, Kiss E, Kapornai K, Daroczi G, Mayer L et al (2008): Association of the neurotrophic tyrosine kinase receptor 3 (NTRK3) gene and childhood-onset mood disorders. *Am J Psychiatry* 165: 610-616.
73. Mercader JM, Saus E, Aguera Z, Bayes M, Boni C, Carreras A et al (2008): Association of NTRK3 and its interaction with NGF suggest an altered cross-regulation of the neurotrophin signaling pathway in eating disorders. *Hum Mol Genet* 17: 1234-1244.
74. Alonso P, Gratacos M, Menchon JM, Segalas C, Gonzalez JR, Labad J et al (2008): Genetic susceptibility to obsessive-compulsive hoarding: the contribution of neurotrophic tyrosine kinase receptor type 3 gene. *Genes Brain Behav* 7: 778-785.
75. Otnaess MK, Djurovic S, Rimol LM, Kulle B, Kahler AK, Jonsson EG et al (2009): Evidence for a possible association of neurotrophin receptor (NTRK-3) gene polymorphisms with hippocampal function and schizophrenia. *Neurobiol Dis* 34: 518-524.
76. Ioannidis JP, Thomas G, Daly MJ (2009): Validating, augmenting and refining genome-wide association signals. *Nat Rev Genet* 10: 318-329.

Chapter 5

A Genome-wide Linkage Scan of Theta Band Activity as a Heritable Endophenotype for Schizophrenia

Submitted

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Abstract

Background Deviant brain oscillations are candidate endophenotypes for schizophrenia. Our aim was to find new quantitative trait loci for brain oscillations and putatively schizophrenia.

Methods We systematically investigated the genetic characteristics of theta, alpha, and beta oscillations at frontal, central, and occipital scalp locations in 25 extended multiplex families affected with schizophrenia using familial correlations, heritability estimates, and segregation analysis. Subsequently, in a genome-wide linkage scan we genotyped seven pedigrees (n=118, including 649 relative pairs) for 6,090 single nucleotide polymorphism markers. Two-point and multipoint variance-component based linkage analyses were performed using MERLIN.

Results Theta activity at occipital sites constituted the most heritable phenotype (h2 of up to 0.55), fitting Mendelian transmission models and was included in a genome-wide scan. Suggestive two-point peaks (empirical $p<0.001$) for theta at occipital sites were found at 13 loci, e.g., 5p15.31, 20p13.

Conclusion Theta activity at occipital sites is the most heritable of the measured frequency bands in line with previous studies. Suggestive linkage peaks were observed, including the locus for DTNBP1. Contrary to expectations EEG was not more powerful than previously investigated cognitive endophenotypes in the same linkage sample.

Introduction

Schizophrenia (MIM# 181500) can be characterized as a highly heritable though complexly inherited disorder.[1,2] As genetic effects are likely to be small impeding the identification of genetic variants and genomic loci for schizophrenia, a combined approach of multiple lines of evidence is needed to further the field of genetic research in schizophrenia. One line of research has focused on alternative indicators of liability, intermediate phenotypes or 'endophenotypes'. Endophenotypes that are heritable, stable, reliable, and are present in the healthy relatives[3] may be used in genetic research to refine the phenotype. The use of endophenotypes facilitates employing quantitative information from all family members and thus the detection of the underlying gene(s) or genetic loci.

Oscillatory brain activity or electroencephalogram (EEG) has a long history in schizophrenia research[4] and represents a promising endophenotype for schizophrenia. EEG in resting state reflects the activity of various circuits of underlying neurons and is correlated to personality and cognitive features.[5] Generally, individuals with schizophrenia display increased low frequency (delta and theta waves)[6] decreased alpha waves, and increased beta (high) frequency activity[7] Slowing of the EEG in schizophrenia has been linked to an impaired subcortical synchronization system including the mesencephalic reticular formation, nucleus reticularis and the thalamus.[8] It has a high heritability,[9-12] good reliability,[13] good stability,[14-16] and is deviant in both patients[7] and relatives.[17-19] Moreover, it may more closely reflect the underlying genetic effects than behavioral task performance, as has been suggested for brain activity phenotypes.[20,21]

Although the heritability of oscillatory brain activity is largely established only few studies have incorporated EEG in a linkage design. Linkage analysis has the advantage of being a hypothesis free method to localize potential genetic variants that have a larger effect size on the trait of interest than genes that may be identified in association analysis. To our knowledge, four studies have performed linkage analyses on evoked EEG, three of which focused on families with alcoholism[22-25] and one on a working memory task in healthy twins.[26] EEG in resting state has been implemented in two genome-wide linkage studies.[27,28] These studies reported linkage on chromosome 20 for low voltage EEG (EEG with reduced alpha rhythms) and significant linkage between beta 2 (16.5–20.0 Hz) activity and a locus on chromosome 4 with a linkage disequilibrium with the *GABRB1* locus. The other frequency bands did not reach significant linkage.

In the present study, we studied the familial correlations, heritability, and pattern of inheritance of oscillatory components on frontal, central, and occipital scalp locations during rest intervals of a P50 task in 139 subjects from 25 extended families with at least one subject affected with schizophrenia. We aimed to identify heritable EEG endophenotypes in order to search for quanti-

tative trait loci (QTLs) that influence variation in oscillatory activity, whether they are novel or replications of schizophrenia loci. With that objective, we performed a genome-wide high-density linkage scan on a subset of families using those traits with heritable characteristics.

Materials and Methods

Participants A total of 181 individuals, including 36 patients and 145 relatives, from 25 multiplex multigenerational pedigrees of Dutch origin were recruited from the general population. Each pedigree comprised at least two members with a diagnosis of schizophrenia or schizoaffective disorder based on the Family Interview for Genetic Studies (FIGS)[29] and at least one member's diagnosis was confirmed by interview. Family size ranged from 2 to 21 relatives with a mean of 7.24 (standard deviation (sd) = 4.93). The sample contained 653 pairings of relatives for whom genetic and diagnostic status was available, which includes all possible pairings within each family: 139 parent-offspring pairs, 165 sib-pairs, 228 second-degree relatives, and 121 third-degree relatives. No loops or consanguineous mating pairs were present. Exclusion criteria were: severe medical or neurological illness; history of closed-head injury; loss of consciousness longer than 30 minutes; history of alcohol abuse within last 6 months; diseases of the central nervous system; history of cerebrovascular accidents, dementia, or delirium; age < 16; or IQ < 70. The sample for linkage analysis consisted of 7 families selected from the 25 families mentioned earlier on the basis of size and information content (as described previously in Aukes, submitted). The average family size in the linkage sample was roughly 17 members (range: 11 to 26) with on average more than three generations (3: 71.4%, 4: 28.6%). Written informed consent was obtained from all participants after complete description of the study. The study was approved by the Medical Ethics Committee of the UMC Utrecht.

Diagnostic assessment Patients were diagnosed by DSM-IV criteria on the basis of the Comprehensive Assessment of Symptoms and History (CASH), a semi-structured diagnostic interview[30] and by retrieving medical records. Psychiatric illness in relatives was assessed by means of the Mini International Neuropsychiatric Interview (MINI-PLUS), a structured clinical interview of DSM axis 1 diagnoses.[31]

EEG measurement A detailed description of the P50 task was given elsewhere.[32] Briefly, subjects were seated in a light and sound attenuated room, and were instructed to keep the eyes closed while counting the number of paired clicks (every 10 seconds (s), 36 in total). EEG's on 32 electrodes were recorded by means of the Active Two System (Biosemi, Amsterdam) and sampled at 2048 Hz. Two additional electrodes in the electrode cap, the CMS (=common mode sense) and DRL (=driven right leg) provided an active ground. We used all (other) electrodes as

a reference, i.e., an average reference.[33] EEG data was analyzed using the software package Brain Vision Analyser (Brain Products, GmbH, Gilching, Germany) and filtered offline with a high-pass filter of 0.1 Hz and a low-pass filter of 40 Hz. Data was re-sampled offline at 256 Hz. We selected 35 4-second inter-trial epochs starting 4 s after the first stimulus until 2 s before the next first stimulus from electrode sites F3, F4, Fz, Cz, O1, O2, and Oz (odd numbers indicate electrodes on the left side of the head, even numbers indicate electrodes on the right side, and 'z' indicates midline). Eye movement artefacts were removed with the method of Gratton et al.[34] Subsequently, we divided the 4 s segments into 1 s segments and performed artefact rejection with a low activity criterion of 0.5 μV and a difference criterion of 100 μV per second. Epochs (1s) were subjected to a Fast Fourier Transformation (FFT) using a Hanning window for calculation of relative power (μV^2) in the following frequency bands: theta: 3-7.5 Hz, alfa: 7.5-12.5 Hz and (low frequency) beta: 12.5-20 Hz. Power was normalized within a window from 3-20 Hz. Frequency spectra were averaged across segments within each electrode location. Subjects with less than 20 s of artefact-free data were excluded from analysis (n= 3). Because having the eyes open or closed could affect the power bands and coherence[33] we excluded participants who did not follow the instructions to close the eyes during the task based on visual inspection of the electro-oculogram (n = 24).

Genotyping Genomic DNA was extracted from EDTA blood samples using standard salting out procedure. We typed 6,090 SNPs distributed evenly across the genome using Illumina Infinium HumanLinkage-12 arrays (Illumina, San Diego, CA). The mean and median interval between markers is 0.44 Mb (0.58 cM) and 0.32 Mb (0.35 cM), respectively. The SNPs were genotyped using the Illumina BeadArray™ technology on an Illumina BeadStation following the manufacturer's protocol.

Error detection All SNPs were examined for their resulting quality; SNPs with a low signal or too wide clusters were excluded (n=80). PLINK (version 1.05)[35] was used to check the data for gender errors. We checked for Mendelian inconsistencies using the program Pedcheck[36] and identified problem genotypes using MERLIN.[37] Sporadic genotyping errors (n = 38) were removed within the families. One case of non-paternity was identified and resolved by introducing a dummy father. Also, we identified a non-affected individual with a maternally inherited uniparental disomy (UPD) of chromosome 22, which was removed from the analyses for this chromosome only.

Data analysis Prior to data analysis we performed an ln-transformation to reduce kurtosis and skewness of the data, as the variance component method (see below) may be vulnerable to high levels of kurtosis in the trait distribution.[38] All analyses were corrected for age and sex.

Familial correlations We obtained standardized residuals corrected for age and sex from regression analysis and used these for calculating parent-offspring (PO) and sib-sib (SS) correlations using the FCOR module in S.A.G.E. (SAGE, v6.0).[39]

Heritability Heritability analysis was performed using the variance component-based program SOLAR (version 4.1, Southwest Foundation for Biomedical Research, San Antonio, Texas).[40] It measures the narrow sense heritability defined as the phenotypic variance explained by additive genetic factors. Components of variance were estimated by maximum likelihood including variation caused by the covariates age and sex, if significant, in a multistep procedure. The significance of the heritability estimate was computed by comparing the polygenic model with the significant covariates to a sporadic model that had the genetic component removed.

Segregation analysis For commingling and segregation analysis we followed the method described in more detail previously[32] using S.A.G.E. In brief, commingling analysis provides guidance in choosing initial parameters for segregation analysis. It fits and compares mixtures of up to three normal distributions. We investigated both major gene models and oligogenic models. In subsequent segregation analysis we determined if a major gene is involved in the trait's variability. Segregation analysis estimates maximum likelihoods of transmission probabilities and allele frequencies for various genetic and environmental models. In a general model all parameters are set to be arbitrary providing the best adjustment to the data. This model serves as a reference model to which all other models are compared using chi-square tests. When no clear discrimination between models could be made, Akaike's An Information Criterion (AIC) was used.

Linkage analysis To test for non-parametric linkage between SNPs and endophenotypes, univariate two-point and multipoint linkage analyses were performed using the variance-components (VC) models implemented in MERLIN and the companion program to MERLIN, MINX for the X chromosome. Variance-components linkage analysis estimates the proportion of variance that can be explained by an underlying QTL, by examining the expected genetic covariances between relatives as a function of their IBD relationships at a given SNP. In MERLIN, IBD is calculated using the Lander-Green algorithm with sparse gene flow trees and background covariance is assumed to be entirely due to additive genetic effects. Variance components were estimated by maximum-likelihood analysis of ln-transformed data along with fixed effects for sex and age. In multipoint analysis we used two centimorgan (cM) spacing. We performed sensitivity analyses to insure that the peak LOD scores were not due to the effect of a single family. With regard to strong dependence among some of the phenotypes, Bonferroni adjustment for multiple testing may be too stringent and imply loss of power. Therefore, we provide here only uncorrected nominal results, which were compared with simulation thresholds.

Simulation To protect against false positives and adjust for possible biases induced by factors such as outliers or non-random missing data, we calculated empirical p-values. We performed two-point linkage analyses on 1000 simulated data sets, in which original phenotypic data were retained, while new genotypes were simulated with MERLIN under the null hypothesis of no linkage. The allele frequencies, marker spacing, and missing data pattern were kept the same. For multipoint linkage simulation we applied the thresholds for suggestive and significant LOD scores of 1.9 and 3.3 as suggested by Lander & Kruglyak.[41]

Table 1 Sample description for unaffected and affected individuals, the total sample, and the linkage sample

	Unaffected		Affected		Total sample		Linkage sample [a]	
# Male (%)	99	39 (39.4%)	12	10 (83.3%)	111	49 (44.1%)	58	27 (46.6%)
	n	M (sd)	n	M (sd)	n	M (sd)	n	M (sd)
Age	99	46.5 (15.9)	12	39.9 (13.3)	111	45.8 (15.7)	58	41.6 (16.7)
Theta F3	99	7.96 (5.95)	12	8.52 (6.72)	111	8.02 (6.01)	58	8.12 (5.89)
Theta Fz	99	8.62 (6.05)	12	8.16 (7.26)	111	8.57 (6.16)	58	9.38 (6.30)
Theta F4	99	8.12 (6.15)	12	9.15 (7.75)	111	8.24 (6.31)	58	8.99 (6.60)
Theta Cz	99	8.41 (6.13)	12	10.36 (7.87)	111	8.62 (6.33)	58	9.38 (6.45)
Theta O1	97	6.25 (4.80)	12	6.96 (6.48)	109	6.33 (4.98)	56	6.58 (5.15)
Theta Oz	94	7.13 (5.01)	12	8.17 (7.40)	106	7.25 (5.30)	55	7.29 (5.29)
Theta O2	97	6.75 (5.11)	11	7.87 (8.33)	108	6.87 (5.48)	55	6.48 (5.01)
Alfa F3	99	10.43 (6.92)	12	12.00 (3.44)	111	10.60 (6.64)	58	11.37 (8.01)
Alfa Fz	99	10.38 (6.67)	12	12.46 (5.30)	111	10.61 (6.55)	58	10.96 (7.85)
Alfa F4	99	10.32 (6.18)	12	12.20 (5.78)	111	10.52 (6.14)	58	10.82 (7.27)
Alfa Cz	99	9.79 (6.10)	12	9.68 (3.63)	111	9.78 (5.87)	58	10.15 (7.18)
Alfa O1	97	12.36 (7.11)	12	13.84 (5.39)	109	12.53 (6.94)	56	12.80 (8.34)
Alfa Oz	94	11.83 (7.35)	12	12.60 (5.42)	106	11.92 (7.14)	55	12.48 (8.63)
Alfa O2	97	12.41 (7.62)	11	13.56 (5.55)	108	12.53 (7.42)	55	13.58 (8.87)
Beta F3	99	2.91 (2.12)	12	2.80 (2.89)	111	2.90 (2.20)	58	2.69 (1.90)
Beta Fz	99	2.52 (2.04)	12	2.74 (2.66)	111	2.55 (2.11)	58	2.16 (1.67)
Beta F4	99	2.87 (2.02)	12	2.28 (1.97)	111	2.81 (2.01)	58	2.49 (1.92)
Beta Cz	99	3.02 (2.54)	12	3.10 (2.44)	111	3.03 (2.52)	58	2.66 (2.63)
Beta O1	97	2.80 (2.05)	12	2.63 (2.76)	109	2.78 (2.13)	56	2.85 (2.27)
Beta Oz	94	2.65 (1.86)	12	2.64 (2.35)	106	2.65 (1.91)	55	2.64 (1.97)
Beta O2	97	2.46 (1.88)	11	2.52 (2.38)	108	2.47 (1.92)	55	2.46 (2.00)

Note: Affected and unaffected individuals did not significantly differ from each other on any of the EEG measures.

[a] The linkage sample (7 pedigrees) was selected from total sample (25 pedigrees) and contains both affected and non-affected individuals.

Table 2 Minimum and maximum correlations within and between the three frequency bands

		Theta	Alpha	Beta
Theta	Min	0.69**		
	Max	0.87**		
Alpha	Min	-0.30**	0.67**	
	Max	-0.58**	0.91**	
Beta	Min	0.20*	0.00	0.55**
	Max	0.52**	-0.31**	0.91**

** Correlation is significant at the 0.01 level (2-tailed).

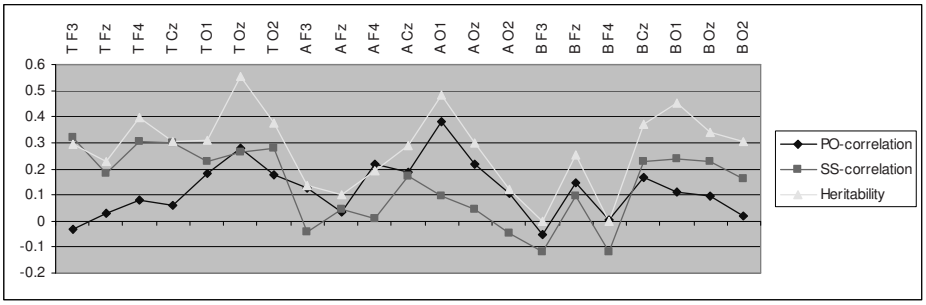
* Correlation is significant at the 0.05 level (2-tailed).

Results

Table 1 provides a description of the data for the theta, alpha, and beta power spectra on the seven electrode sites (Fz, F3, F4, Cz, Oz, O1, and O2). There were no significant differences between the affected and unaffected individuals (Table 1). All patients received anti-psychotics in contrast to the relatives. Age had moderate effects on alpha at F3, O2, and Cz (F(df) = 1.77(52), 1.81(50), 1.64(52); p= 0.02, 0.02, 0.03, respectively). Only beta power was different among the sexes with females having higher beta power than males at all leads (F≥8.96 (1), p<0.003). Theta band showed strong negative correlations with alpha at all scalp locations and positive correlations with beta (Table 2). Alpha and beta power were moderately correlated.

Heritability The parent-offspring correlations, sib-sib correlations, and heritability estimates for each of the power bands at the various electrode sites are given in Figure 1 (details in Supplementary Table S1). Generally, heritability was higher on occipital sites for all power bands and

Figure 1 Parent-offspring (PO), sib-sib (SS) correlations and heritability estimates for theta (T), alpha (A), and beta (B) frequency bands at the seven electrode sites



Note: Exact estimates and standard errors are given in Supplementary Table S1

highest for theta (h2=0.55 at theta Oz). The theta frequency band showed a mean heritability over the electrodes of 0.35, ranging from 0.23 to 0.55. Alpha and beta frequency bands were less heritable with average estimates of 0.23 and 0.25, respectively.

Segregation analysis In order to include traits with the highest genetic load and to reduce the number of tests for further segregation and linkage analysis we selected traits based on the threshold of a PO correlation above 0.2, or higher than 0.1 when SS correlation was above 0.2 (as in Aukes et al.)[32], with the additional criterion of a heritability above 0.3, resulting in the selection of theta O1, theta Oz, theta O2, alpha O1, beta O1, and beta Cz. In the commingling analysis, all selected traits fitted to a two or three means distribution model (two means: theta Oz; theta O2; alpha O1; three means: theta O1; beta O1) suggestive of an underlying genetic model,[42] except for beta Cz, which was therefore omitted from further segregation analysis (for commingling results see supplementary Table S2). In the segregation analysis, the non-transmission (environmental) model could be rejected for all three theta measures (Table 3). A Mendelian model provided the best fit for theta at O1 and Oz, resembling dominant transmission for theta Oz, while theta O1 showed a heterozygote advantage. Both models explained 56% of the variance (using the method as previously described),[32] which fits to the estimated heritability of theta Oz, though is somewhat higher than the heritability of theta O1. Theta O2 best fitted a homogeneous general model, implying complex inheritance. Alpha O1 and beta O1 resulted in a

Table 3 Segregation analyses: likelihood estimates (Akaike's An Information criterion [AIC] model fit) for selected traits

	Model	d	L	Hom no transmission	Hom mendelian	Hom general	τ_{AB}-free	General (Ref.)
Theta O1	FPMM	three	3	305.87*[a]	**301.97***[c]	303.86*[b]	303.97*[a]	300.68*[b]
Theta Oz	FPMM	two	2	307.82*[a]	**294.03***[c]	298.00*[b]	295.46*[b]	295.93*[b]
Theta O2	FPMM	two	2	303.40*[a]	300.07*[c]	**299.83*[b]**	301.43*[b]	300.64*[b]
Alpha O1	D	two	-	**289.11**	292.25*[c]	292.89	294.00	294.45
Beta O1	FPMM	three	3	**306.92**	324.00*[a,b]	311.61[1]	322.98*[a,b]	312.86[1]

Note: in bold are the best fitted models.

Abbreviations: L: number of loci fitted in FPMM; d: number of distributions; Hom: homogeneous; Ref.: reference model; FPMM: Finite Polygenic Mixed Model; D: class D regression model.

*[a] The corresponding -2likelihood is significantly different from the reference model at $p < 0.05$.

*[b] The corresponding -2likelihood is significantly different from the no transmission model at $p < 0.05$.

*[c] Not comparable to the no transmission model in a χ^2 test, because the degrees of freedom = 0.

[1] Flat likelihood or infinite standard error.

Table 4 Two-point linkage peaks for theta O1, theta O2, and theta Oz

	Theta O1					Theta O2					Theta Oz				
Cytogenic band	Marker	Position[a]	LOD	Emp p	Peds	Marker	Position[a]	LOD	Emp p	Peds	Marker	Position[a]	LOD	Emp p	Peds
3p12.3-11.2	rs1562499	104.2	**2.92**	**0.00077**	1,2,3,6,7	rs9826824	109.7	**1.87**	**0.00027**	3,5,6,7	rs13038	111.7	**2.47**	**0.00014**	1,2,3,5,6,7
3q29						rs2986	209.8	**1.66**	**0.00057**	2,3,4,5,6,7					
5p15.31	rs4524477	19.9	**3.66**	**0.00014**	all						rs4524477	19.9	1.52	0.00306	1,3,4,5,6,7
6p22.3						rs74111	34.9	**1.7**	**0.00049**	2,3,4,6,7					
6q14.3-16.1	rs1325182	93.4	**3.29**	**0.00035**	all	rs2493964	101.3	**1.57**	**0.00078**	3,4,5,6,7	rs1325182	93.4	1.53	0.00301	1,2,3,4,5,6
7p22.1											rs13438509	13.4	**1.97**	**0.00074**	1,2,3,4,5,6
11p14.3-14.2	rs7480892	43.3	2.62	0.00151	1,2,3,5,6	rs2593688	37.6	**1.99**	**0.00017**	4,5,6,7					
15q26.2	rs2639197	115.3	**2.83**	**0.00094**	1,2,3,4,6	rs288394	120.8	**1.82**	**0.00032**	2,4,5,6	rs288394	120.8	**1.93**	**0.00086**	1,2,3,4,5,6
16p13.2-13.12	rs1158123	32.5	**3.43**	**0.00025**	all						rs1019141	20	1.58	0.0026	1,2,3,4,5,7
19p13.3	rs197198	1.7	1.81	0.0079	2,3,5,7						rs197198	1.7	**2.02**	**0.00015**	1,3,4,5,7
19p13.2	rs13535	28.9	**3.36**	**0.0003**	1,3,4,5,6	rs1044250	26.5	**1.54**	**0.00089**	1,3,4,5,6	rs13535	28.9	**2.38**	**0.00019**	1,3,4,5,6
20p13	rs6080305	0.5	2.78	0.00105	1,2,3,5,6,7	rs6080305	0.5	**2.34**	**0.00004**	2,3,4,5,6	rs6080305	0.5	1.53	0.00298	2,3,4,5,6,7
20q13.13	rs718630	77.9	**3.42**	**0.00025**	1,2,3,6,7										

Note: Results are shown for SNPs with empirical p-values below the suggestive threshold of p<0.001 (bolded), and results for the other measures with a LOD>1.5 if nearby. If multiple suggestive peaks were close to each other, only the highest peak is given.

Abbreviations: Emp p: empirical p-values; Peds: pedigrees that contributed positively to the LOD-score.

[a] Map distance in cM correspond with the deCode genetic map, sex-averaged distances.

best fit to an environmental model. Specifications of all model parameters are given in Supplementary Table S3.

Linkage analysis In our sample, the average marker heterozygosity was 44.5%, the average minor allele frequency was 35%, and the missing rate was 0.014%. Linkage analysis was performed on the three measures that fitted to transmission models in segregation analysis: theta O1, O2, and Oz. Based on 1000 simulations, the LOD-thresholds for suggestive ($p<0.001$) and significant ($p<0.00005$) empirical two-point p-values were for theta O1: 2.8, 4.07; theta O2: 1.51, 2.32; and theta Oz: 1.88, 2.75, respectively. In multipoint linkage analysis, we did not observe LOD-scores higher than 1 (Supplementary Figure S1). However, in the two-point analysis, we observed 28 suggestive peaks for the three occipital theta phenotypes with empirical p-values below the suggestive threshold of $p<0.001$ in the following regions: 3p12-11, 3q29, 5p15, 6p22, 6q14-16, 7p22, 11p14, 15q26, 16p13, 19p13, 19p13, 20p13, and 20q13 (Table 4 and Supplementary Figure S2). As expected, the results were very similar for these highly correlated phenotypes; the genetic correlations for these three measures converged to 1 (data not shown). The most notable two-point linkage region appeared at 5p15.31 for theta O1 (LOD 3.66, empirical $p = 0.0001$), and for theta Oz (LOD= 1.52, empirical $p= 0.003$) at the same SNP rs4524477. This was also the only region that stood out in multipoint linkage (Figure 2), although LOD scores were trivial (LOD = 0.47). The most suggestive finding for theta O2 was observed at 20p13 for SNP rs6080305 (LOD 2.34, empirical $p= 0.00004$), with peaks for the other occipital sites at the same marker (theta O1: LOD 2.78, empirical $p= 0.001$; theta Oz: 1.53, empirical $p= 0.003$). Suggestive peaks for all three measures were further observed at 3p12.3-11.2 (SNPs rs1562499, rs9826824, and rs13038), 15q26.2 (SNPs rs2639197 and rs288394), and 19p13.2 (SNPs rs13535 and rs1044250), which are all overlapping with or close (~1-10Mb) to loci for schizophrenia and/or related electrophysiological phenotypes (Supplementary Table S4). Notably, the suggestive finding for theta O2 at 6p22.3 (rs74111, LOD= 1.7, empirical $p=0.0005$) lies within the Dysbindin gene (DTNBP1).

Discussion

In this study we systematically investigated the genetic background of EEG endophenotypes for schizophrenia in extended families affected with schizophrenia. We found that theta band activity and activity at occipital sites constituted the most heritable phenotypes with a heritability of 0.55 for theta at Oz. Subsequently, we observed several suggestive two-point linkage peaks for theta band activity at three occipital sites in the following regions: 3p12-11, 3q29, 5p15, 6p22,

6q14-16, 7p22, 11p14, 15q26, 16p13, 19p13, 19p13, 20p13, and 20q13, including the DTNBP1 gene, corroborating its involvement in schizophrenia and neurocognitive endophenotypes.

Heritability study Our estimates of familial correlations and proportions of heritability of several frequency bands at frontal, central, and occipital scalp locations support earlier findings that low frequency oscillatory activity (theta) recorded over occipital scalp locations is a heritable endophenotype.[9,10] Alpha and beta at occipital sites showed moderate familial correlations and heritability though did not fit to genetic models of transmission. Theta at two of the three occipital sites fitted to simpler patterns of inheritance resembling Mendelian inheritance and may therefore be particularly promising as endophenotypes for schizophrenia to be used in genetic research. Our findings fit with the notion that genetic influences on the human EEG may be band-specific[10] and different neural networks and neurotransmitter systems induce activity in the different frequency ranges.[4] Contrary to expectations, heritability estimates were not higher than estimates for cognitive endophenotypes measured in the same sample previously,[32] suggesting that theta oscillatory phenotypes may not reflect the underlying genetic effects more closely than behavioral measures.

In mammals and humans theta activity has been implicated in memory and working memory (for review see Uhlhaas et al.).[4] Indeed, we found a genetic correlation between theta activity at O2 and Oz, and spatial working memory (SWM) measured in the same individuals[32] (O2-SWM= -0.44, Oz-SWM= -0.25, O1-SWM= 0.049). It is thus of importance to further investigate the dynamics of theta activity during the inter-trial intervals of the P50 task for its putative underlying cognitive processes. Previously, theta band during the time-locked gating response of the P50 task was also shown to be heritable,[43] which suggests that heritability of theta is not limited to non-stationary frequency activity.

Linkage study Our most notable finding is the suggestive peak for theta O2 within the Dysbindin gene (DTNBP1 at 6p22.3), within a linkage region for schizophrenia.[44] Dysbindin has been associated with schizophrenia multiple times[45-47] and is thought to be involved in glutamate release. Notably, it has also shown significant associations to other endophenotypes for schizophrenia, such as early visual perception,[48] prefrontal brain function,[49] memory,[50] cognition, and IQ.[51,52] DTNBP1 is thus a likely candidate gene residing in the 6p22.3 region to affect brain oscillatory activity and schizophrenia.

In the genome-wide linkage scan, four (3p11.2-12.3, 3q29, 6p22.3 and 16p13.12) of the twelve suggestive linkage regions overlapped with loci for schizophrenia (5 if bipolar disorder is included [20p13]).[44,53-55] Moreover, several regions were located close to peaks for related endophenotypes. This overlap increases the likelihood that genes in these regions contribute both to theta activity at occipital sites and schizophrenia. The peak at 20q13.13 for theta O1 is located

within the vicinity of the marker linked to low-voltage EEG at 20q13.2-q13.3.[27] The linkage peak at 3p11.2-12.3 lies within a locus for schizophrenia[54] and in between loci for N100 response on a visual selective attention task.[24] Peaks that were not located in the vicinity to previous findings for schizophrenia (e.g. 11p14.3) may, if well replicated, harbor genes with only specific effects on oscillatory activity.

Of interest, our suggestive findings at 15q26.2 (SNPs rs2639197 and rs288394) are ~7.5 Mb removed from NTRK3 (neurotrophic tyrosine kinase receptor), a potential susceptibility gene for schizophrenia (Aukes, submitted) through a suggestive linkage finding for spatial working memory at 15q26.1. Although located somewhat remotely, the vicinity of the linkage region for spatial working memory fits with the genetic correlation between theta activity at O2 and Oz with spatial working memory mentioned above, suggesting that these traits may share genetic loci. NTRK3 may through its effects on nervous system development and myelination[56] affect low frequency oscillations, spatial working memory, and schizophrenia.

We did not find suggestive linkage peaks near two candidate genes for theta band activity: catechol-O-methyl transferase (COMT) at chromosome 22, which has been associated with delta and theta activity in schizophrenia patients,[19] or the cholinergic muscarinic receptor gene (CHRM2) at chromosome 7 for low frequency visual evoked brain oscillations.[25]

Strengths and limitations A limiting factor is, as in most linkage studies, that we could only detect loci for susceptibility genes with a large effect due to limited power as a result of multiple testing and limited sample size. This may bias the results towards zero particularly in multipoint linkage analysis, which depends on the distribution of the traits within the families. Therefore, while there may be an increase in power in QTL analysis of endophenotypes, our study illustrates some of the complexity of incorporating endophenotypes in genetic research and the need for large and well documented samples of both family data and population-based cohorts. Nevertheless, we were able to identify several loci of interest that were supported by previous findings and analyses in independent samples. The strength of our study lies in the combination of analytical techniques. Such a full-range approach is desired when dealing with complex phenotypes and when genetic effects are likely to be small. We first selected endophenotypes on the basis of their heritable characteristics followed by segregation analysis and finally localizing QTLs for oscillatory activity.

Summarizing, our results provide further support for the suitability of theta oscillatory activity at occipital sites as an endophenotype for schizophrenia and reveal several suggestive loci for this trait. Despite the low power of our study, most of our suggestive two-point linkage peaks are likely to harbor candidate genes for occipital theta and schizophrenia, as was supported by simu-

lation and by overlap with loci for schizophrenia, related endophenotypes, and with one of the best candidate genes for schizophrenia, DTNBP1.

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Web Resources

http://pngu.mgh.harvard.edu/purcell/plink/

http://www.schizophreniaforum.org/res/sczgene

Online Supplementary Material

Supplementary Table S1 Heritability, parent-offspring (PO) and sib-sib (SS) correlations for theta, alpha, and beta frequency bands at the seven electrode sites

Trait	n	H2r	p	Res kurt	n	PO-corr	p	n	SS-corr	p
Theta_Oz	106	0.553 ± 0.264	0.009	-0.769	44	0.279 ± 0.15	0.085	59	0.265 ± 0.156	0.107
Theta_O2	108	0.376 ± 0.267	0.06	-0.221	46	0.177 ± 0.165	0.306	60	0.279 ± 0.16	0.098
Theta_O1	109	0.31 ± 0.23	0.072	-0.824	47	0.18 ± 0.14	0.216	62	0.228 ± 0.155	0.157
Theta_Fz	111	0.229 ± 0.185	0.082	-0.738	49	0.031 ± 0.153	0.846	67	0.183 ± 0.148	0.229
Theta_F4	111	0.395 ± 0.197	0.016	-0.293	49	0.082 ± 0.151	0.596	67	0.307 ± 0.157	0.067
Theta_F3	111	0.293 ± 0.189	0.037	-0.561	49	-0.032 ± 0.171	0.854	67	0.321 ± 0.153	0.051
Theta_Cz	111	0.303 ± 0.198	0.043	-0.658	49	0.058 ± 0.164	0.731	67	0.298 ± 0.155	0.071
Beta_Oz	106	0.34 ± 0.208	0.041	-0.49	44	0.093 ± 0.146	0.535	59	0.226 ± 0.16	0.172
Beta_O2	108	0.306 ± 0.208	0.055	-0.024	46	0.019 ± 0.136	0.889	60	0.161 ± 0.153	0.306
Beta_O1	109	0.453 ± 0.234	0.023	-0.073	47	0.111 ± 0.142	0.446	62	0.24 ± 0.158	0.146
Beta_Fz	111	0.254 ± 0.183	0.055	-0.127	49	0.144 ± 0.152	0.361	67	0.094 ± 0.145	0.519
Beta_F4	111	0	0.5	-0.35	49	0.004 ± 0.132	0.977	67	-0.116 ± 0.1	0.252
Beta_F3	111	0	0.5	0.711	49	-0.053 ± 0.111	0.641	67	-0.118 ± 0.111	0.292
Beta_Cz	111	0.369 ± 0.177	0.009	-0.308	49	0.165 ± 0.147	0.28	67	0.226 ± 0.15	0.146
Alfa_Oz	106	0.3 ± 0.239	0.07	1.268	44	0.217 ± 0.128	0.107	59	0.044 ± 0.143	0.759
Alfa_O2	108	0.122 ± 0.232	0.282	0.43	46	0.107 ± 0.137	0.449	60	-0.045 ± 0.135	0.74
Alfa_O1	109	0.483 ± 0.227	0.006	1.378	47	0.383 ± 0.117	0.004	62	0.096 ± 0.145	0.513
Alfa_Fz	111	0.1 ± 0.162	0.249	0.132	49	0.035 ± 0.133	0.794	67	0.043 ± 0.132	0.748
Alfa_F4	111	0.193 ± 0.192	0.122	0.725	49	0.219 ± 0.121	0.083	67	0.011 ± 0.125	0.932
Alfa_F3	111	0.137 ± 0.158	0.161	1.204	49	0.126 ± 0.137	0.373	67	-0.043 ± 0.126	0.733
Alfa_Cz	111	0.291 ± 0.179	0.028	0.382	49	0.185 ± 0.145	0.221	67	0.171 ± 0.153	0.275

Abbreviations: p = p-value; O: occipital; F: frontal; C: central; corr: correlation; Res Kurt: residual kurtosis.

Supplementary Table S2: Commingling analyses: model fit of class-D regressive models and Finite Polygenic Mixed Models (FPMM) for selected traits

							Distribution comparison					
							Vs two means			Vs three means		
Trait	Model	L	D	-2lnL	AIC	N	χ^2	df	p	χ^2	df	p
TO1	Class-D	-	one	309.7	317.7	4	11.31	2	0.004	12.82	2	0.002
			two	298.4	310.4	6				1.52	0	nc
			three	296.9	308.9	6						
	FPMM	3	one	311.4	319.4	4	15.07	2	0.001	19.56	3	0.000
			two	296.4	308.4	6				4.49	1	0.034
			three	291.9	**305.9**	7						
TOz	Class-D	-	one	299.8	307.8	4	1.65	2	0.437	1.87	3	0.599
			two	298.2	310.2	6				0.22	1	0.640
			three	298.0	312.0	7						
	FPMM	2	one	305.1	313.1	4	9.34	2	0.009	10.21	3	0.017
			two	295.8	**307.8**	6				0.87	1	0.350
			three	294.9	308.9	7						
TO2	Class-D	-	one	302.9	310.9	4	1.15	2	0.563	4.33	3	0.228
			two	301.8	313.8	6				3.19	1	0.074
			three	298.6	312.6	7						
	FPMM	2	one	305.1	313.1	4	13.65	2	0.001	15.21	3	0.002
			two	291.4	**303.4**	6				1.56	1	0.212
			three	289.8	303.8	7						
AO1	Class-D	-	one	282.4	290.37 [a]	4	5.26	2	0.072	5.37	3	0.147
			two	277.1	**289.1**	6				0.11	1	0.739
			three	277.0	291.0	7						
	FPMM	3	one	287.6	293.6	3	5.34	2	0.069	6.48	3	0.090
			two	282.3	292.3	5				1.14	1	0.286
			three	281.2	293.2	6						
BO1	Class-D	-	one	310.3	318.3	4	1.93	2	0.380	3.98	3	0.263
			two	308.3	320.3	6				2.05	1	0.152
			three	306.3	320.3	7						
	FPMM	3	one	314.0	322.0	4	1.6	1	0.206	19.11	3	0.001
			two	312.4	322.4	5				17.51	2	0.001
			three	294.9	**308.9**	7						
BCz	Class-D	-	one	306.1	**314.1**	4	0.72	2	0.698	0.91	2	0.633
			two	305.3	317.3	6				0.19	0	nc
			three	305.1	317.1	6						
	FPMM	3	one	476.0	482.03 [a]	3	166.25	2	0.001	170.09	2	0.001
			two	309.8	319.79 [a]	5				3.84	0	nc
			three	306.0	316.0	5						

Abbreviations: Vs: versus; L: number of loci modeled in FPMM; d: number of distributions; -2lnL: -2log likelihood; AIC: An Information Criterion; N: number of estimated parameters; χ^2: chi square statistic; df: degrees of freedom; T: theta; nc: not comparable; A: alpha; B: beta.

[a] The model resulted in an infinite standard error or maximization problem.

Supplementary Table S3 Final segregation model parameters

Parameter	Parameter estimate ± SE				
	Theta O1	Theta Oz	Theta O2	Alpha O1	Beta O1
Model	FPMM	FPMM	FPMM	D	FPMM
Means	Three	Two	Two	Two	Three
L	3	2	2		3
	hom	hom			
Model	mendelian	mendelian	hom general	hom no transm	hom no transm
Mean (AA)	10.10 ± 0.19	10.91 ± 0.07	10.48 ± 0.04	8.41 ± 0.39	8.64 ± 0.04
Mean (AB)	10.90 ± 0.04	= mean (AA)	= mean (AA)	= mean (AA)	10.08 ± 0.02
Mean (BB)	9.33 ± 0.04	9.15 ± 0.06	9.05 ± 0.05	10.33 ± 0.12	11.15 ± 0.03
Variance	0.02 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.51 ± 0.09	0.00 ± 0[a]
$\sigma_{polygenic}$	0.41 ± 0.04	0.38 ± 0.04	0.56 ± 0.05	-	0.21 ± 0.01
ρPO=ρSS	-	-	-	0.39 ± 0.18	-
q[a]	0.24 ± 0.05	0.26 ± 0.05	0.37 ± 0.05	0.07 ± 0.03	0.52 ± 0.04
τ(AA)	[1]	[1]	0.58 ± 0.39	-	-
τ(AB)	[0.5]	[0.5]	0.53 ± 0.13	-	-
τ(BB)	[0]	[0]	0.11 ± 0.1	-	-

Abbreviations: L: number of loci fitted in FPMM; means: number of distributions; $\sigma_{polygenic}$: residual polygenic variance; ρ_{PO}= ρ_{SS}: residual familial correlations; q_A: allele frequency; τ_{AA}, τ_{AB}, τ_{BB}: transmission probabilities for genotypes AA, AB, BB.

Note: parameters in square brackets are fixed. Means of genotypes AA, AB, BB are on a standardized scale, corrected for age and sex, with mean 10 and standard deviation of 1.

[a] Parameter converged to a bound.

Supplementary Table S4 Two-point linkage peaks for theta O1, theta O2 and theta Oz with corresponding multipoint linkage results, and review of previous genome-wide linkage studies of schizophrenia (SCZ) and bipolar disorder (BIP), meta-analyses of linkage studies (META GWL), and linkage studies of related endophenotypes

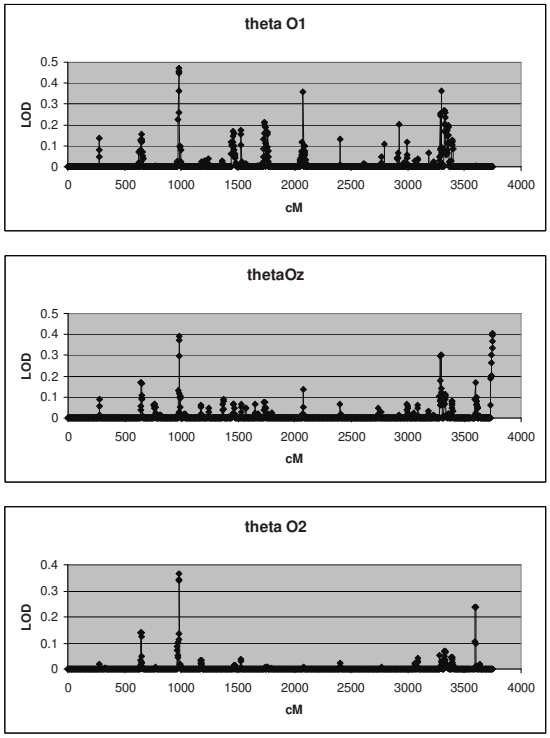
		Two-point results					Multipoint results (LOD)				Previous studies		
Chr	Trait	Marker	Bp	cM	LOD	Emp pointwp	cM	Theta Oz	Theta O2	Theta O1	Linkage SCZ, BIP	META GWL SCZ and related	Electrofysiological endophenotypes
3p12.3	Theta O1	rs1562499	76685083	104.2	**2.92**	**0.00077**	111	0.17	0.14	0.16	SCZ+BIP 68.8mb NPL 2.33; [57]	**SCZ 71.6-120mb Psr 0.04047 Por 0.01140;** [54]	Visual N1 at several electrodes, ~102-119cM, LOD ≤ 2.58; [24]
3p11.2	Theta O2	rs9826824	88462148	109.7	**1.87**	**0.00027**					SCZ 73mb MP_Z 2.33; [58]	SA_MSP 124 cM (Marshfield) 0.04 and BAMSP 0.2; [59]	
	Theta Oz	rs13038	99708041	111.7	**2.47**	**0.00014**					SCZ+BIP 73mb LOD_TP 2.22; [60] SCZ 74mb LOD 1.74; [61] **SCZ 85mb LOD 2.5;** [62] **SCZ 85mb NPL 1.66;** [63] **SCZ+BIP 85.3mb NPL 3.55;** [57] **SCZ+BIP 99mb NPL 3.15;** [57] SCZ 108mb LOD_REC 2.4; [64]		
3q29	Theta O2	rs2986	194013903	209.8	**1.66**	**0.00057**	209	0.00	0.00	0.00	BIP 186.9mb NPL 1.96; [65] BIP 187.8mb LOD_TP 2.66; [66] BIP 190mb LOD 1.94; [67] SCZ 187mb 190mb LOD 2.31 1.95; [68] SCZ+BIP 190.7mb LOD_TP 3.03; [60] **SCZ+BIP 194mb NPL_Z 3.74;** [57] **BIP 194.1mb HLOD 2.0;** [69] **BIP 194.1mb LOD_TP 1.90;** [70] **SCZ+BIP 194.1mb NPL 4.09;** [57] **SCZ 195.6mb LOD 2.6;** [71] **SCZ_BIP 198mb NPL_Z 3.5;** [57] **SCZ 198mb LOD 2.79;** [72]	**SCZ-BIP 209 cM (Marshfield)** **SA_MSP 0.1 and BAMSP 0.3;** [59]	Semantic Priming Paradigm P3 Unprimed O1, 184cM; LOD_VC_MP 3.08;[23]
5p15.31	Theta O1	rs4524477	7616922	19.9	**3.66**	**0.00014**	16-20	0.39	0.37	0.47	BIP 3.2mb LOD_TP 2.77; [66]	-	PZ, C3 and Cz in memory condition, 54-65 cM, LOD 1.89; [26]
	Theta Oz	rs4524477	7616922	19.9	1.52	0.00306					BIP 3.4mb LOD 2.05; [73] **SCZ+SA 5mb NPL 2.18;** [74] BIP 9.2mb LOD_TP 2.12; [75] **SCZ+BIP 9.2mb LOD_TP 1.91;** [60]		
6p22.3	Theta O2	rs74111	15894580	34.9	**1.70**	**0.00049**	38	0.00	0.00	0.03	**SCZ 15-20mb LOD 2.15;** [76] **SCZ 9-36mb HLOD 2.36-3.46;** [45] BIP ~15mb NPL 4.88; [77] BIP 24.6mb ZLOD 2.00; [78] BIP 24.5mb NPL 2.07; [79]	**SCZ 0-16mb GSMA 3.17, 16-43mb GSMA 2.87;** [44] SA_MSP 44 cM (Marshfield), 0.03, BA_MSP 0.04; [59]	-
6q14.3	Theta O1	rs1325182	87487183	93.4	**3.29**	**0.00035**	94	0.00	0.00	0.00	**SCZ 94mb NPL 1.56;** [80]	ADHD 43-90 GSMA 2.78, 90-132mb GSMA 2.73; [81]	P3 (Cz), 91 cM, LOD_VC_MP 1.86; [22]
	Theta Oz	rs1325182	87487183	93.4	1.53	0.00301					**SCZ+BIP 91.3mb NPL 2.7;** [57]	BIP 104-104mb LOD 1.8, 104-112mb LOD 2.7, 104-112mb LOD 4.19; [55]	
6q16.1	Theta O2	rs2493964	95428654	101.3	**1.57**	**0.00078**					BIP 104.8mb LOD 2.95; [82] BIP 104.8mb LOD_TP 2.62; [83] BIP 104.8mb LOD_TP 2.59; [70]		
7p22.1	Theta Oz	rs13438509	6949546	13.4	**1.97**	**0.00074**	11	0.09	0.00	0.01	**SCZ+SA 2mb NPL 2.14;** [84] **BIP 11.6mb HLOD 2.17;** [85]	-	-
11p14.3	Theta O2	rs2593688	22345606	37.6	**1.99**	**0.00017**	38	0.00	0.00	0.02	BIP 36mb LOD_TP 2.53; [70]	**AUT 18-36mb HEGESMA 2.97;** [86]	O2 in memory condition, 57 cM LOD 1.74; [26]
11p14.2	Theta O1	rs7480892	26367162	43.3	2.62	0.00151							
15q26.2	Theta O1	rs2639197	94107957	115.3	**2.83**	**0.00094**	124	0.01	0.00	0.02	**SCZBIP 95mb LOD_REC 2.31;** [87]	-	-
	Theta O2	rs288394	95933425	120.8	**1.82**	**0.00032**					**SCZBIP 97mb NPL 1.93;** [88]		

Supplementary Table S4 (continued)

		Two-point results					Multipoint results (LOD)				Previous studies		
Chr	Trait	Marker	Bp	cM	LOD	Emp pointw p	cM	Theta Oz	Theta O2	Theta O1	Linkage SCZ, BIP	META GWL SCZ and related	Electrofysiological endophenotypes
	Theta Oz	rs288394	95933425	120.8	**1.93**	**0.00086**					BIP 97mb HLOD 1.96; [65]		
16p13.2	Theta Oz	rs1019141	7658287	20.0	1.58	0.00260	17	0.00	0.00	0.11	BIP ~1mb NPL_MP 1.99; [89]	**SCZ 13.2-51.5mb Psr 0.01775 Por 0.15808;** [54]	**Visual N1 at several electrodes, ~18 cM (Marshfield), LOD 2.16;** [24]
16p13.12	Theta O1	rs1158123	13616365	32.5	**3.43**	**0.00025**					BIP 6.2mb LOD_TP 2.23; [70] BIP 6.2mb LOD 4.07; [90] BIP 12.3mb NPL 3.3; [91] BIP 13.9mb LOD 2.3; [92] BIP 15.6-23.0mb NPL 2.57; [93] BIP 17.0mb NPL 2.73; [73] **SCZBIP 18.2-18.5mb MOD 4.05 & 3.66;** [87] BIP 18.9bm LOD 2.3; [92] **SCZ 20mb NPL 2.12;** [94] **SCZ 23.5mb MLS 2.52;** [95]		
19p13.3	Theta O1	rs197198	1456873	1.7	1.81	0.00790	4	0.01	0.00	0.00	**Schizotypy & SCZ, 19p13.3, emperical genomewide p that both traits were NPL>1 0.04;** [96]	-	-
19p13.3	Theta Oz	rs197198	1456873	1.7	**2.02**	**0.00015**					**SCZ 3mb LOD_DOM 2.04;** [97]		
19p13.2	Theta O2	rs1044250	8342163	26.5	**1.54**	**0.00089**	26	0.00	0.00	0.00	**SCZ 9mb NPL 1.58;** [80]		
19p13.2	Theta O1	rs13535	9315172	28.9	**3.36**	**0.00030**					**SCZBIP 12mb LOD 1.85;** [98]		
19p13.2	Theta Oz	rs13535	9315172	28.9	**2.38**	**0.00019**					BIP 14.5mb NPL 2.06; [99] **SCZ 15mb NPL 1.94;** [100]		
20p13	Theta O1	rs6080305	172970	0.5	2.78	0.00105	0	0.10	0.05	0.05	BIP 7.4mb NPL 2.11; [89]	**BIP 0.603-1.0mb LOD 3.8 4mb LOD 1.91;** [55]	**Semantic Priming Paradigm P3 Nonsense C4, 0 cM, LOD 2.46;** [23]
20p13	Theta O2	rs6080305	172970	0.5	**2.34**	**0.00004**					**SCZ 8.9mb LOD_MP 2.34;** [58]	SCZ 7-21mb GSMA 2.74; [44]	
20p13	Theta Oz	rs6080305	172970	0.5	1.53	0.00298					BIP 10.1mb NPL 2.8; [91] BIP 10.1mb LOD 3.23; [82]		
20q13.13	Theta O1	rs718630	48544662	77.9	**3.42**	**0.00025**	78	0.02	0.01	0.20	**SCZ 39mb NPL 1.54;** [94] **SCZ 43.3mb MLS 2.7;** [95] BIP ~50mb LOD_MP 1.98; [101] **SCZ 55.6mb MLS 2.39;** [95] **SCZ 57mb NPL 2.1;** [102]	BIP 58mb LOD 2.5; [55]	**Cz, O1, Fz and F4 in memory condition, 57-62 and 83 cM, LOD 2.29;** [26] **Low voltage EEG, 20q13.2-q13.3, max_LOD 3.13;** [27]

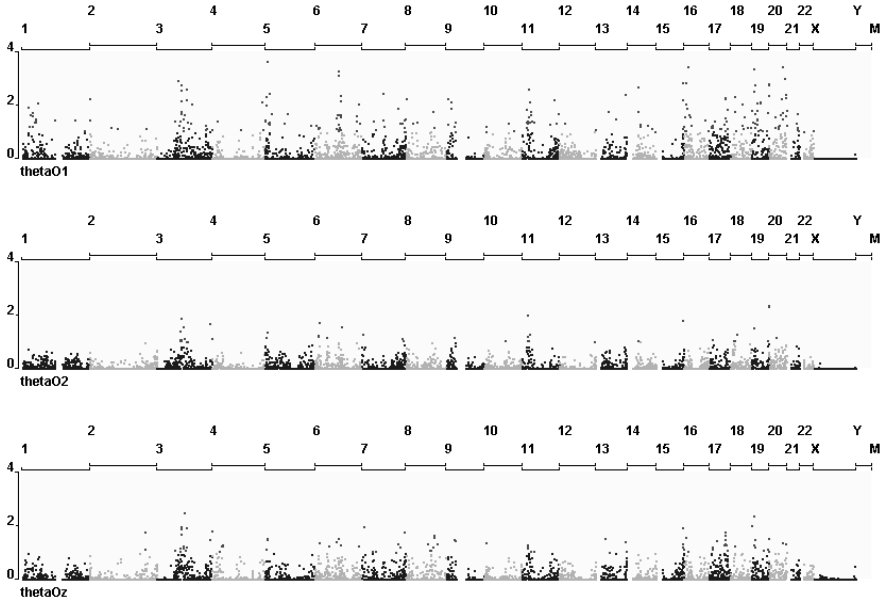
Note: Lod scores and empirical p-values in bold are suggestive findings; the studies in bold are linkage studies for schizophrenia within a 10 Mb distance of the suggestive marker(s). Abbreviations: Chr: chromosome; cM: centimorgan map distance corresponding with the deCode genetic map, sex-averaged distances; Emp pointw p: empirical point-wise p-value; MP: multipoint; NPL: nonparametric linkage; REC: recessive; TP: two-point.

Supplementary Figure S1 Multipoint linkage plots for theta at O1, O2, and Oz



Note: The x-axis corresponds to cumulative cM on the deCode genetic map, sex-averaged distances, over all 22 autosomes and the X chromosome.

Supplementary Figure S2 Genome-wide two-point linkage plots for theta at O1, O2, and Oz



Note: The x-axis represents sex-averaged cM distance on the deCode genetic map, on the consecutive chromosomes (number indicated on top); the y-axis represents LOD scores.

References

1. Kendler KS, Diehl SR (1993): The genetics of schizophrenia: a current, genetic-epidemiologic perspective. *Schizophr Bull* 19: 261-285.
2. Sullivan PF (2005): The genetics of schizophrenia. *PLoS Med* 2: e212.
3. Gottesman II, Gould TD (2003): The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 160: 636-645.
4. Uhlhaas PJ, Haenschel C, Nikolic D, Singer W (2008): The role of oscillations and synchrony in cortical networks and their putative relevance for the pathophysiology of schizophrenia. *Schizophr Bull* 34: 927-943.
5. Vogel F, Schalt E (1979): The electroencephalogram (EEG) as a research tool in human behavior genetics: psychological examinations in healthy males with various inherited EEG variants. III. Interpretation of the results. *Hum Genet* 47: 81-111.
6. Doege K, Bates AT, White TP, Das D, Boks MP, Liddle PF (2009): Reduced event-related low frequency EEG activity in schizophrenia during an auditory oddball task. *Psychophysiology* 46: 566-577.
7. Boutros NN, Arfken C, Galderisi S, Warrick J, Pratt G, Iacono W (2008): The status of spectral EEG abnormality as a diagnostic test for schizophrenia. *Schizophr Res* 99: 225-237.
8. Kirino E (2004): Correlation between P300 and EEG rhythm in schizophrenia. *Clin EEG Neurosci* 35: 137-146.
9. Vogel F (1970): The genetic basis of the normal human electroencephalogram (EEG). *Humangenetik* 10: 91-114.
10. Zietsch BP, Hansen JL, Hansell NK, Geffen GM, Martin NG, Wright MJ (2007): Common and specific genetic influences on EEG power bands delta, theta, alpha, and beta. *Biol Psychol* 75: 154-164.
11. Stassen HH, Coppola R, Gottesman II, Torrey EF, Kuny S, Rickler KC et al (1999): EEG differences in monozygotic twins discordant and concordant for schizophrenia. *Psychophysiology* 36: 109-117.
12. van Beijsterveldt CE, van Baal GC (2002): Twin and family studies of the human electroencephalogram: a review and a meta-analysis. *Biol Psychol* 61: 111-138.
13. Lund TR, Sponheim SR, Iacono WG, Clementz BA (1995): Internal consistency reliability of resting EEG power spectra in schizophrenic and normal subjects. *Psychophysiology* 32: 66-71.
14. Pollock VE, Schneider LS, Lyness SA (1991): Reliability of topographic quantitative EEG amplitude in healthy late-middle-aged and elderly subjects. *Electroencephalogr Clin Neurophysiol* 79: 20-26.
15. Salinsky MC, Oken BS, Morehead L (1991): Test-retest reliability in EEG frequency analysis. *Electroencephalogr Clin Neurophysiol* 79: 382-392.
16. Lifshitz K, Lee KL, Susswein S (1987): Long-term replicability of EEG spectra and auditory evoked potentials in schizophrenic and normal subjects. *Neuropsychobiology* 18: 205-211.
17. Itil TM (1977): Qualitative and quantitative EEG findings in schizophrenia. *Schizophr Bull* 3: 61-79.
18. Alfimova M, Uvarova L (2003): Cognitive peculiarities in relatives of schizophrenic and schizoaffective patients: heritability and resting EEG-correlates. *Int J Psychophysiol* 49: 201-216.
19. Venables NC, Bernat EM, Sponheim SR (2008): Genetic and Disorder-Specific Aspects of Resting State EEG Abnormalities in Schizophrenia. *Schizophr Bull* .
20. Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE et al (2001): Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A* 98: 6917-6922.
21. Green AE, Munafo MR, DeYoung CG, Fossella JA, Fan J, Gray JR (2008): Using genetic data in cognitive neuroscience: from growing pains to genuine insights. *Nat Rev Neurosci* 9: 710-720.
22. Begleiter H, Porjesz B, Reich T, Edenberg HJ, Goate A, Blangero J et al (1998): Quantitative trait loci analysis of human event-related brain potentials: P3 voltage. *Electroencephalogr Clin Neurophysiol* 108: 244-250.
23. Almasy L, Porjesz B, Blangero J, Goate A, Edenberg HJ, Chorlian DB et al (2001): Genetics of event-related brain potentials in response to a semantic priming paradigm in families with a history of alcoholism. *Am J Hum Genet* 68: 128-135.

24. Porjesz B, Begleiter H, Wang K, Almasy L, Chorlian DB, Stimus AT et al (2002): Linkage and linkage disequilibrium mapping of ERP and EEG phenotypes. *Biol Psychol* 61: 229-248.
25. Jones KA, Porjesz B, Almasy L, Bierut L, Goate A, Wang JC et al (2004): Linkage and linkage disequilibrium of evoked EEG oscillations with CHRM2 receptor gene polymorphisms: implications for human brain dynamics and cognition. *Int J Psychophysiol* 53: 75-90.
26. Hansell NK, Medland SE, Ferreira MA, Geffen GM, Zhu G, Montgomery GW et al (2006): Linkage analyses of event-related potential slow wave phenotypes recorded in a working memory task. *Behav Genet* 36: 29-44.
27. Steinlein O, Anokhin A, Yping M, Schalt E, Vogel F (1992): Localization of a gene for the human low-voltage EEG on 20q and genetic heterogeneity. *Genomics* 12: 69-73.
28. Porjesz B, Almasy L, Edenberg HJ, Wang K, Chorlian DB, Foroud T et al (2002): Linkage disequilibrium between the beta frequency of the human EEG and a GABAA receptor gene locus. *Proc Natl Acad Sci U S A* 99: 3729-3733.
29. National Institute of Mental Health. Molecular Genetics Initiative: Family Interview for Genetic Studies. 1991. Bethesda, MD, National Institute of Mental Health. Ref Type: Report
30. Andreasen NC, Flaum M, Arndt S (1992): The Comprehensive Assessment of Symptoms and History (CASH). An instrument for assessing diagnosis and psychopathology. *Arch Gen Psychiatry* 49: 615-623.
31. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E et al (1998): The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* 59 Suppl 20: 22-33.
32. Aukes MF, Alizadeh BZ, Sitskoorn MM, Selten JP, Sinke RJ, Kemner C et al (2008): Finding Suitable Phenotypes for Genetic Studies of Schizophrenia: Heritability and Segregation Analysis. *Biol Psychiatry* .
33. Nunez PL, Silberstein RB, Shi Z, Carpenter MR, Srinivasan R, Tucker DM et al (1999): EEG coherency II: experimental comparisons of multiple measures. *Clin Neurophysiol* 110: 469-486.
34. Gratton G, Coles MG, Donchin E (1983): A new method for off-line removal of ocular artifact. *Electroencephalogr Clin Neurophysiol* 55: 468-484.
35. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D et al (2007): PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81: 559-575.
36. O'connell JR, Weeks DE (1998): PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 63: 259-266.
37. Abecasis GR, Cherny SS, Cookson WO, Cardon LR (2002): Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 30: 97-101.
38. Allison DB, Neale MC, Zannolli R, Schork NJ, Amos CI, Blangero J (1999): Testing the robustness of the likelihood-ratio test in a variance-component quantitative-trait loci-mapping procedure. *Am J Hum Genet* 65: 531-544.
39. S.A.G.E. Statistical Analysis for Genetic Epidemiology 6.0.0 Computer program package available from the Department of Epidemiology and Biostatistics. 2008. Metro Health Campus, Case Western Reserve University, Cleveland, Rammelkamp Center for Education and Research. Ref Type: Computer Program
40. Almasy L, Blangero J (1998): Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 62: 1198-1211.
41. Lander E, Kruglyak L (1995): Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11: 241-247.
42. Kwon JM, Boehnke M, Burns TL, Moll PP (1990): Commingling and segregation analyses: comparison of results from a simulation study of a quantitative trait. *Genet Epidemiol* 7: 57-68.
43. Hong LE, Summerfelt A, Mitchell BD, McMahon RP, Wonodi I, Buchanan RW et al (2008): Sensory gating endophenotype based on its neural oscillatory pattern and heritability estimate. *Arch Gen Psychiatry* 65: 1008-1016.
44. Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I et al (2003): Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 73: 34-48.
45. Straub RE, MacLean CJ, Ma Y, Webb BT, Myakishev MV, Harris-Kerr C et al (2002): Genome-wide scans of three independent sets of 90 Irish multiplex schizophrenia families and follow-up of

selected regions in all families provides evidence for multiple susceptibility genes. *Mol Psychiatry* 7: 542-559.
46. Harrison PJ, Weinberger DR (2005): Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. *Mol Psychiatry* 10: 40-68.
47. Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ et al (2008): Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. *Nat Genet* 40: 827-834.
48. Donohoe G, Morris DW, De SP, Magno E, Montesi JL, Garavan HP et al (2008): Early visual processing deficits in dysbindin-associated schizophrenia. *Biol Psychiatry* 63: 484-489.
49. Fallgatter AJ, Herrmann MJ, Hohoff C, Ehlis AC, Jarczok TA, Freitag CM et al (2006): DTNBP1 (dysbindin) gene variants modulate prefrontal brain function in healthy individuals. *Neuropsychopharmacology* 31: 2002-2010.
50. Hashimoto R, Noguchi H, Hori H, Nakabayashi T, Suzuki T, Iwata N et al (2009): A genetic variation in the dysbindin gene (DTNBP1) is associated with memory performance in healthy controls. *World J Biol Psychiatry* : 1-8.
51. Burdick KE, Lencz T, Funke B, Finn CT, Szeszko PR, Kane JM et al (2006): Genetic variation in DTNBP1 influences general cognitive ability. *Hum Mol Genet* 15: 1563-1568.
52. Luciano M, Miyajima F, Lind PA, Bates TC, Horan M, Harris SE et al (2009): Variation in the dysbindin gene and normal cognitive function in three independent population samples. *Genes Brain Behav* 8: 218-227.
53. Badner JA, Gershon ES (2002): Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 7: 405-411.
54. Ng MY, Levinson DF, Faraone SV, Suarez BK, DeLisi LE, Arinami T et al (2008): Meta-analysis of 32 genome-wide linkage studies of schizophrenia. *Mol Psychiatry* .
55. McQueen MB, Devlin B, Faraone SV, Nimgaonkar VL, Sklar P, Smoller JW et al (2005): Combined analysis from eleven linkage studies of bipolar disorder provides strong evidence of susceptibility loci on chromosomes 6q and 8q. *Am J Hum Genet* 77: 582-595.
56. Cosgaya JM, Chan JR, Shooter EM (2002): The neurotrophin receptor p75NTR as a positive modulator of myelination. *Science* 298: 1245-1248.
57. Bailer U, Leisch F, Meszaros K, Lenzinger E, Willinger U, Strobl R et al (2002): Genome scan for susceptibility loci for schizophrenia and bipolar disorder. *Biol Psychiatry* 52: 40-52.
58. Suarez BK, Duan J, Sanders AR, Hinrichs AL, Jin CH, Hou C et al (2006): Genomewide linkage scan of 409 European-ancestry and African American families with schizophrenia: suggestive evidence of linkage at 8p23.3-p21.2 and 11p13.1-q14.1 in the combined sample. *Am J Hum Genet* 78: 315-333.
59. Badner JA, Gershon ES (2002): Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* 7: 405-411.
60. Klei L, Bacanu SA, Myles-Worsley M, Galke B, Xie W, Tiobech J et al (2005): Linkage analysis of a completely ascertained sample of familial schizophrenics and bipolars from Palau, Micronesia. *Hum Genet* 117: 349-356.
61. Faraone SV, Skol AD, Tsuang DW, Young KA, Haverstock SL, Prabhudesai S et al (2005): Genome scan of schizophrenia families in a large Veterans Affairs Cooperative Study sample: evidence for linkage to 18p11.32 and for racial heterogeneity on chromosomes 6 and 14. *Am J Med Genet B Neuropsychiatr Genet* 139: 91-100.
62. Gurling HM, Kalsi G, Brynjolfson J, Sigmundsson T, Sherrington R, Mankoo BS et al (2001): Genomewide genetic linkage analysis confirms the presence of susceptibility loci for schizophrenia, on chromosomes 1q32.2, 5q33.2, and 8p21-22 and provides support for linkage to schizophrenia, on chromosomes 11q23.3-24 and 20q12.1-11.23. *Am J Hum Genet* 68: 661-673.
63. Faraone SV, Hwu HG, Liu CM, Chen WJ, Tsuang MM, Liu SK et al (2006): Genome scan of Han Chinese schizophrenia families from Taiwan: confirmation of linkage to 10q22.3. *Am J Psychiatry* 163: 1760-1766.
64. Brzustowicz LM, Hodgkinson KA, Chow EW, Honer WG, Bassett AS (2000): Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. *Science* 288: 678-682.
65. Herzberg I, Jasinska A, Garcia J, Jawaheer D, Service S, Kremeyer B et al (2006): Convergent linkage evidence from two Latin-American population isolates supports the presence of a susceptibility locus for bipolar disorder in 5q31-34. *Hum Mol Genet* 15: 3146-3153.

66. Kelsoe JR, Spence MA, Loetscher E, Foguet M, Sadovnick AD, Remick RA et al (2001): A genome survey indicates a possible susceptibility locus for bipolar disorder on chromosome 22. *Proc Natl Acad Sci U S A* 98: 585-590.
67. Zandi PP, Badner JA, Steele J, Willour VL, Miao K, MacKinnon DF et al (2007): Genome-wide linkage scan of 98 bipolar pedigrees and analysis of clinical covariates. *Mol Psychiatry* 12: 630-639.
68. DeLisi LE, Mesen A, Rodriguez C, Bertheau A, Laprade B, Llach M et al (2002): Genome-wide scan for linkage to schizophrenia in a Spanish-origin cohort from Costa Rica. *Am J Med Genet* 114: 497-508.
69. Curtis D, Kalsi G, Brynjolfsson J, McInnis M, O'Neill J, Smyth C et al (2003): Genome scan of pedigrees multiply affected with bipolar disorder provides further support for the presence of a susceptibility locus on chromosome 12q23-q24, and suggests the presence of additional loci on 1p and 1q. *Psychiatr Genet* 13: 77-84.
70. Ewald H, Flint T, Kruse TA, Mors O (2002): A genome-wide scan shows significant linkage between bipolar disorder and chromosome 12q24.3 and suggestive linkage to chromosomes 1p22-21, 4p16, 6q14-22, 10q26 and 16p13.3. *Mol Psychiatry* 7: 734-744.
71. Devlin B, Bacanu SA, Roeder K, Reimherr F, Wender P, Galke B et al (2002): Genome-wide multipoint linkage analyses of multiplex schizophrenia pedigrees from the oceanic nation of Palau. *Mol Psychiatry* 7: 689-694.
72. Paunio T, Ekelund J, Varilo T, Parker A, Hovatta I, Turunen JA et al (2001): Genome-wide scan in a nationwide study sample of schizophrenia families in Finland reveals susceptibility loci on chromosomes 2q and 5q. *Hum Mol Genet* 10: 3037-3048.
73. Cheng R, Juo SH, Loth JE, Nee J, Iossifov I, Blumenthal R et al (2006): Genome-wide linkage scan in a large bipolar disorder sample from the National Institute of Mental Health genetics initiative suggests putative loci for bipolar disorder, psychosis, suicide, and panic disorder. *Mol Psychiatry* 11: 252-260.
74. Shaw SH, Kelly M, Smith AB, Shields G, Hopkins PJ, Loftus J et al (1998): A genome-wide search for schizophrenia susceptibility genes. *Am J Med Genet* 81: 364-376.
75. Adams LJ, Mitchell PB, Fielder SL, Rosso A, Donald JA, Schofield PR (1998): A susceptibility locus for bipolar affective disorder on chromosome 4q35. *Am J Hum Genet* 62: 1084-1091.
76. Schwab SG, Hallmayer J, Albus M, Lerer B, Eckstein GN, Borrmann M et al (2000): A genome-wide autosomal screen for schizophrenia susceptibility loci in 71 families with affected siblings: support for loci on chromosome 10p and 6. *Mol Psychiatry* 5: 638-649.
77. Marcheco-Teruel B, Flint TJ, Wikman FP, Torralbas M, Gonzalez L, Blanco L et al (2006): A genome-wide linkage search for bipolar disorder susceptibility loci in a large and complex pedigree from the eastern part of Cuba. *Am J Med Genet B Neuropsychiatr Genet* 141B: 833-843.
78. Turecki G, Grof P, Grof E, D'Souza V, Lebuis L, Marineau C et al (2001): Mapping susceptibility genes for bipolar disorder: a pharmacogenetic approach based on excellent response to lithium. *Mol Psychiatry* 6: 570-578.
79. Avramopoulos D, Lasseter VK, Fallin MD, Wolyniec PS, McGrath JA, Nestadt G et al (2007): Stage II follow-up on a linkage scan for bipolar disorder in the Ashkenazim provides suggestive evidence for chromosome 12p and the GRIN2B gene. *Genet Med* 9: 745-751.
80. Kaufmann CA, Suarez B, Malaspina D, Pepple J, Svrakic D, Markel PD et al (1998): NIMH Genetics Initiative Millenium Schizophrenia Consortium: linkage analysis of African-American pedigrees. *Am J Med Genet* 81: 282-289.
81. Zhou K, Dempfle A, rcos-Burgos M, Bakker SC, Banaschewski T, Biederman J et al (2008): Meta-analysis of genome-wide linkage scans of attention deficit hyperactivity disorder. *Am J Med Genet B Neuropsychiatr Genet* 147B: 1392-1398.
82. Goes FS, Zandi PP, Miao K, McMahon FJ, Steele J, Willour VL et al (2007): Mood-incongruent psychotic features in bipolar disorder: familial aggregation and suggestive linkage to 2p11-q14 and 13q21-33. *Am J Psychiatry* 164: 236-247.
83. Lambert D, Middle F, Hamshere ML, Segurado R, Raybould R, Corvin A et al (2005): Stage 2 of the Wellcome Trust UK-Irish bipolar affective disorder sibling-pair genome screen: evidence for linkage on chromosomes 6q16-q21, 4q12-q21, 9p21, 10p14-p12 and 18q22. *Mol Psychiatry* 10: 831-841.

84. Lerer B, Segman RH, Hamdan A, Kanyas K, Karni O, Kohn Y et al (2003): Genome scan of Arab Israeli families maps a schizophrenia susceptibility gene to chromosome 6q23 and supports a locus at chromosome 10q24. *Mol Psychiatry* 8: 488-498.
85. Friddle C, Koskela R, Ranade K, Hebert J, Cargill M, Clark CD et al (2000): Full-genome scan for linkage in 50 families segregating the bipolar affective disease phenotype. *Am J Hum Genet* 66: 205-215.
86. Trikalinos TA, Karvouni A, Zintzaras E, Ylisaukko-oja T, Peltonen L, Jarvela I et al (2006): A heterogeneity-based genome search meta-analysis for autism-spectrum disorders. *Mol Psychiatry* 11: 29-36.
87. Maziade M, Roy MA, Chagnon YC, Cliche D, Fournier JP, Montgrain N et al (2005): Shared and specific susceptibility loci for schizophrenia and bipolar disorder: a dense genome scan in Eastern Quebec families. *Mol Psychiatry* 10: 486-499.
88. Vazza G, Bertolin C, Scudellaro E, Vettori A, Boaretto F, Rampinelli S et al (2007): Genome-wide scan supports the existence of a susceptibility locus for schizophrenia and bipolar disorder on chromosome 15q26. *Mol Psychiatry* 12: 87-93.
89. Cassidy F, Zhao C, Badger J, Claffey E, Dobrin S, Roche S et al (2007): Genome-wide scan of bipolar disorder and investigation of population stratification effects on linkage: support for susceptibility loci at 4q21, 7q36, 9p21, 12q24, 14q24, and 16p13. *Am J Med Genet B Neuropsychiatr Genet* 144B: 791-801.
90. Jones I, Hamshere M, Nangle JM, Bennett P, Green E, Heron J et al (2007): Bipolar affective puerperal psychosis: genome-wide significant evidence for linkage to chromosome 16. *Am J Psychiatry* 164: 1099-1104.
91. McInnis MG, Dick DM, Willour VL, Avramopoulos D, MacKinnon DF, Simpson SG et al (2003): Genome-wide scan and conditional analysis in bipolar disorder: evidence for genomic interaction in the National Institute of Mental Health genetics initiative bipolar pedigrees. *Biol Psychiatry* 54: 1265-1273.
92. Dick DM, Foroud T, Edenberg HJ, Miller M, Bowman E, Rau NL et al (2002): Apparent replication of suggestive linkage on chromosome 16 in the NIMH genetics initiative bipolar pedigrees. *Am J Med Genet* 114: 407-412.
93. Cichon S, Schmidt-Wolf G, Schumacher J, Muller DJ, Hurter M, Schulze TG et al (2001): A possible susceptibility locus for bipolar affective disorder in chromosomal region 10q25--q26. *Mol Psychiatry* 6: 342-349.
94. Williams NM, Norton N, Williams H, Ekholm B, Hamshere ML, Lindblom Y et al (2003): A systematic genomewide linkage study in 353 sib pairs with schizophrenia. *Am J Hum Genet* 73: 1355-1367.
95. Lindholm E, Ekholm B, Shaw S, Jalonen P, Johansson G, Pettersson U et al (2001): A schizophrenia-susceptibility locus at 6q25, in one of the world's largest reported pedigrees. *Am J Hum Genet* 69: 96-105.
96. Fanous AH, Neale MC, Gardner CO, Webb BT, Straub RE, O'Neill FA et al (2007): Significant correlation in linkage signals from genome-wide scans of schizophrenia and schizotypy. *Mol Psychiatry* 12: 958-965.
97. Wijsman EM, Rosenthal EA, Hall D, Blundell ML, Sobin C, Heath SC et al (2003): Genome-wide scan in a large complex pedigree with predominantly male schizophrenics from the island of Kosrae: evidence for linkage to chromosome 2q. *Mol Psychiatry* 8: 695-705, 643.
98. Hamshere ML, Bennett P, Williams N, Segurado R, Cardno A, Norton N et al (2005): Genomewide linkage scan in schizoaffective disorder: significant evidence for linkage at 1q42 close to DISC1, and suggestive evidence at 22q11 and 19p13. *Arch Gen Psychiatry* 62: 1081-1088.
99. Fallin MD, Lasseter VK, Wolyniec PS, McGrath JA, Nestadt G, Valle D et al (2004): Genomewide linkage scan for bipolar-disorder susceptibility loci among Ashkenazi Jewish families. *Am J Hum Genet* 75: 204-219.
100. Faraone SV, Matise T, Svrakic D, Pepple J, Malaspina D, Suarez B et al (1998): Genome scan of European-American schizophrenia pedigrees: results of the NIMH Genetics Initiative and Millennium Consortium. *Am J Med Genet* 81: 290-295.
101. Park N, Juo SH, Cheng R, Liu J, Loth JE, Lilliston B et al (2004): Linkage analysis of psychosis in bipolar pedigrees suggests novel putative loci for bipolar disorder and shared susceptibility with schizophrenia. *Mol Psychiatry* 9: 1091-1099.

102. Garver DL, Holcomb J, Mapua FM, Wilson R, Barnes B (2001): Schizophrenia spectrum disorders: an autosomal-wide scan in multiplex pedigrees. *Schizophr Res* 52: 145-160.

Chapter 6

Summary and Discussion

This thesis on the genetics of cognitive endophenotypes in schizophrenia - a family-based study - describes the search for heritable endophenotypes for genetic research in schizophrenia. The main objective of these studies was to unravel the genetic characteristics of promising endophenotypes for schizophrenia. The first part of this thesis focused on investigating the genetic characteristics of the endophenotypes. The second part focused on the implementation of candidate endophenotypes in a genome-wide linkage scan, including the search for positional candidate genes for schizophrenia.

Briefly, it was shown that: only 5 of 13 cognitive candidate endophenotypes revealed moderate heritable characteristics (**Chapter 2**); both shared and distinct genetic effects contribute to these heritable endophenotypes (**Chapter 3**); these endophenotypes are linked with suggestive evidence to a number of quantitative trait loci (QTLs) (**Chapter 4**); several potential candidate genes for schizophrenia are located within these loci (**Chapter 4**); particularly NTRK3 is a susceptibility gene for schizophrenia (**Chapter 4**); and occipital theta is a heritable phenotype that was linked with suggestive evidence to several genomic loci (**Chapter 5**).

Below, first, a summary of each chapter is given, followed by several methodological considerations. Next, the findings of this thesis are discussed, including outlines for future research. A figure summarizing the study design and analyses performed throughout the chapters is given in Chapter 4 (page 110).

Summary

With the aim of identifying constructive endophenotypes for genetic research in schizophrenia, the first study, described in **chapter 2**, investigated the heritable characteristics of 13 candidate endophenotypes for schizophrenia (discussed in **chapter 1**). It was reasoned that for endophenotypes to be useful in genetic research it would be beneficial to have a mode of transmission that is simpler than that of schizophrenia itself, i.e., the endophenotypes should more closely reflect the underlying genetic effects. Familial correlations were calculated for 13 selected endophenotypes in twenty-five multigenerational families that were multiply affected with schizophrenia, followed by heritability analysis, and segregation analysis. The results showed that only 5 of 13 endophenotypes were moderately correlated between family members, i.e., sensorimotor gating, openness, verbal fluency, early visual perception, and spatial working memory, with equivalent heritability estimates (37%–54%). The eight endophenotypes with low familial correlations appeared to be ones that had previously shown conflicting or few heritability estimates. In the segregation analysis, two of the five heritable endophenotypes, i.e., sensorimotor gating and openness, revealed a simpler mode of inheritance, resembling a dominant pattern of transmission.

Verbal fluency, early visual perception, and spatial working memory reflected polygenic or multifactorial effects. Concluding, sensorimotor gating and openness appeared to be promising candidate endophenotypes for genetic research in schizophrenia on the basis of their simpler mode of inheritance. Moreover, not all candidate endophenotypes are heritable in all populations and one should be cautious about incorporating endophenotypes in genetic research, as they may not carry the advantage of a simpler mode of inheritance.

Chapter 3 focussed on investigating the genetic relationships among the five endophenotypes that were shown to be heritable in **chapter 2,** i.e., sensorimotor gating, openness, verbal fluency, early visual perception, and spatial working memory. A sixth trait, intelligence, was added to these analyses for being a promising endophenotype and for being correlated to the other traits. First Pearson's correlations were calculated, followed by estimation of the genetic and environmental contributions to the variance shared between the five heritable endophenotypes and intelligence. The results demonstrated significant correlations among spatial working memory, verbal fluency, and intelligence and between early visual perception and spatial working memory. The correlation between spatial working memory and intelligence could be mainly attributed to genetic factors, implying that overlapping genetic effects contribute to individual differences in spatial working memory and intelligence. Environmental correlations were observed between verbal fluency and openness and between verbal fluency and spatial working memory. In contrast, two other candidate endophenotypes, i.e., sensorimotor gating and openness, appear to be relatively separate heritable entities, showing few genetic or environmental correlations with verbal fluency, spatial working memory, early visual perception, or intelligence. Interestingly, sensorimotor gating and openness were the traits that fitted to simpler modes of inheritance in **chapter 2**. Therefore they seem more likely to originate from distinct and possibly fewer genetic factors. These findings thus support the hypothesis that multiple genes are shared among intelligence, related cognitive measures, and schizophrenia, while a smaller number of independent genetic factors may contribute to distinct endophenotypes, such as sensorimotor gating and openness. Performance on individual tests and their underlying factors seem to be only partly correlated, indicating relatively distinct dimensions of cognitive impairment, as follows from a multifactorial polygenetic model of schizophrenia. Intelligence may be a promising endophenotype for genetic research in schizophrenia, even though the underlying genetic mechanism may still be complex. Sensorimotor gating and openness appeared to represent separate genetic entities with simpler inheritance patterns and may therefore augment the detection of separate genetic pathways contributing to schizophrenia.

In the study described in **chapter 4,** endophenotypes were linked to genotypes. In order to search for QTLs that influence variation in the cognitive endophenotypes, a genome-wide linkage analysis was performed using the six cognitive endophenotypes that were shown to be heritable (**Chapter 2**) and partly genetically correlated (**Chapter 3**). Approximately 6,000 markers (single nucleotide polymorphisms; SNPs) distributed over the genome were genotyped in 91 individuals from seven affected families. Three regions of interest emerged from the multipoint analysis, i.e., 8q21-24 for early visual perception and 16q21-22 and 17p13 for openness. Also, suggestive evidence for several two-point peaks was obtained for sensorimotor gating, verbal fluency, spatial working memory and IQ, e.g., on 2q22.1, 2q37.1, 5q33-34, 9q31-33, and 15q26.1. Most of these loci overlapped or closely bordered to previous linkage findings for schizophrenia, related endophenotypes, or other psychiatric disorders, such as bipolar disorder. Based on the assumption that endophenotypes share genetic factors with schizophrenia, it follows that genes for schizophrenia should reside under (some of the) true linkage peaks for endophenotypes. Therefore, functional interrelations among the genes that were located underneath the linkage peaks as well as shared biological pathways with known candidate genes for schizophrenia were investigated through prioritization analysis. Subsequently, prioritized genes were examined for differential expression in post-mortem brain tissue of schizophrenia patients compared to matched control subjects in published and online datasets. Two of the four prioritized and expressed genes were then found to be associated with schizophrenia in a sample of 758 cases and 676 controls subjects, i.e., EIF4E2, and particularly, NTRK3. In order to test whether the association between NTRK3 and schizophrenia is likely to be true, the same analyses were performed in two larger independent case-control sets (1,172/1,378 and 921/954 cases/controls). Indeed, SNPs in NTRK3 were associated with schizophrenia in both replication samples, even more so, further strengthening the likelihood that this gene is involved in schizophrenia. Summarizing, this study illustrates that endophenotypes, just as the schizophrenia phenotype itself, are complex traits that need large study samples and large informative families to show evidence of linkage. Even so, we were able to identify a potential susceptibility gene for schizophrenia by integration of the classical positional cloning approach with the use of carefully selected endophenotypes and expression profiling datasets, augmenting the depth of studying endophenotypes and schizophrenia.

In the study described in **chapter 5**, the genetic characteristics and potential usefulness for genetic research was investigated for oscillatory brain activity (electroencephalogram; EEG). EEG may more closely reflect the underlying genetic effects than behavioural task performance, as has been suggested for other brain activity phenotypes.[1,2] Oscillatory activity was derived from the resting intervals during the P50 task. Theta activity (3-7.5 Hz) at three occipital sites showed

moderate familial correlations (0.18-0.28) and equivalent heritability estimates (31-55%). In the segregation analysis, theta activity at two of the three occipital sites fitted best to Mendelian transmission models. This trait was therefore selected for a subsequent high-density genome-wide linkage analysis. Two-point peaks with empirical p-values below the suggestive threshold of 0.001 were observed for occipital theta at 13 loci, including 3p12-11, 3q29, 5p15, 6p22, 6q14-16, 7p22, 11p14, 15q26, 16p13, 19p13, 19p13, 20p13, and 20q13. As with the cognitive measures (**Chapter 4**), several peaks overlapped with loci that had previously been linked to schizophrenia. One of the SNPs that showed suggestive evidence of linkage was located within the DTNBP1 gene, a strong candidate gene for schizophrenia that has been implicated in cognitive functioning as well. Given these findings, it can be concluded that theta activity at occipital sites is a suitable endophenotype for schizophrenia on the basis of its heritable characteristics. Contrary, alpha and beta band activity seemed largely affected by environmental factors, particularly at frontal and central leads. Although power was low in the linkage study, potential loci for occipital theta activity were observed, including the DTNBP1 candidate gene for schizophrenia. Altogether, oscillatory activity did not perform better than the cognitive endophenotypes measured previously in the same sample (**Chapters 2 and 3**), suggesting that theta oscillatory endophenotypes may not reflect more closely the underlying genetic effects than behavioural measures.

Methodological considerations

Several methodological considerations extend beyond the separate chapters and are discussed here. Primarily, the findings described above should be replicated in other types of populations. Heritability estimates, segregation patterns, and linkage regions all heavily depend on the population studied, in our case multigenerational pedigrees affected with schizophrenia.

A limitation of the studies described in this thesis is their limited sample size. For example, because of the low power, bivariate heritability analysis yielded large standard errors. Nevertheless, in all studies we were able to combine a range of methods that converged to a consistent pattern of results fitting with theoretical pathogenic models and previous findings.

The present studies were based on the key assumption that endophenotypes are genetically related to schizophrenia and are therefore modulated by genes that also affect schizophrenia. We have not tested this overlap between the endophenotypes and schizophrenia ourselves, though made an initial selection of endophenotypes using previous findings supporting such overlap, as described in the introduction (**Chapter 1**). Particularly the identification of candidate genes was

based on the assumption of genetic overlap and should be reconsidered when this assumption turns out to be false.

Cognitive endophenotypes, including several personality measures, were the core subject of this thesis. The inclusion of endophenotypes was based on the available literature at the start of this project and feasibility of experimental setup. Therefore, we may have missed endophenotypes, such as meta-cognition, emotional traits, or imaging phenotypes, which may be promising for their heritable and 'endophenotypic' characteristics for implementation in genetic research.

The strength of these studies lies in the combination of analytic methods. The convergent results of different methodologies support our findings and allow a critical evaluation of several candidate endophenotypes for schizophrenia. Additional strengths of these studies are the detailed measurement of 13 endophenotypes of which we could select the most heritable ones, limiting the number of tests performed. Also, the use of multigenerational, multiply affected pedigrees is favourable for it is potentially more informative in revealing major gene action and QTLs. Heritability estimates are less likely to be inflated by shared environmental effects as in first-degree relatives.

Discussion

The results described in this thesis support the endophenotypic approach in psychiatric research. It follows from the findings summarised above that careful investigation and selection of endophenotypes should precede implementation in genetic research. For example, more than half of the candidate endophenotypes included in this study (**Chapters 2 and 5**) lack the heritable characteristics required for useful implementation in genetic research. Additionally, only few traits, i.e., sensorimotor gating, openness, and occipital theta, revealed a simpler mode of inheritance, a characteristic that is warranted for endophenotypes, for a smaller number of genes may affect these traits. Such genes may be of greater effect, rendering the traits more suitable for genetic research. At the same time, the univariate non-parametric linkage analyses (Chapters 4 and 5) revealed each of the heritable endophenotypes to perform reasonably well considering the small sample size. Possibly, additional analyses, such as parametric or multivariate linkage analysis, may be able to reveal whether, respectively, traits with a simpler segregation pattern or combined correlated traits perform better than single complex traits in univariate analysis.

The findings in this thesis demonstrate a dynamic pattern of correlations among the endophenotypes (**Chapter 3**) that is difficult though necessary to disentangle when the specific and overlapping genetic contributions are the focus of study. Larger studies that incorporate multiple covariates or stratified analyses may be able to further delineate these complex phenotypic net-

works. Similarly, endophenotypes are not and need not be specific for schizophrenia. Phenotyping beyond the current clinical boundaries may advance unravelling these intricate networks and identification of biological pathways and shared risk factors for several related psychiatric disorders.

The studied endophenotypes appear to be complex heritable traits and heterogeneous entities, raising the question of whether quantitative endophenotypic traits live up to their expectations. It would be of interest to compare the findings to a qualitative trait such as schizophrenia diagnosis, which is however not possible because of low number of patients in the present sample. Other studies suggest endophenotypes may perform as well[3] or better[4] than the qualitative disease trait. Our results showed each of the heritable endophenotypes to have potential in genetic research. Openness and early visual perception were stronger in multipoint linkage, where the other traits clearly lacked power. However, most of the suggestive linkage peaks for verbal fluency, spatial working memory, sensorimotor gating, IQ, and theta oscillations were of interest as shown by simulation, overlap with loci for schizophrenia,[5,6] and location of genes of interest.

Summarising, the selection of suitable and heritable endophenotypes and their subsequent implementation in a family-study has shown to augment genetic research in schizophrenia by identifying a susceptibility gene for schizophrenia. If replicated, the loci observed for the endophenotypes and the potential susceptibility genes for schizophrenia within these loci may contribute to finding mechanisms responsible for schizophrenia. These studies are an example of how phenotype analysis in pedigrees, linkage mapping, gene expression data in brain tissue, and association testing in cases and controls can be used jointly in order to study the genetic basis for complex neuropsychiatric traits.

Future perspectives

The search for well-defined, heritable, reliable, stable, and thus useful endophenotypes is an ongoing laborious task. For example, a key issue that deserves more attention is the reliability of endophenotypes. A reliability below 80%, which is common for cognitive tasks, may introduce such error that counteracts the genetic effect one is looking for.[7] Identification of endophenotypes that have a high reliability will augment the dissection of underlying genetic and environmental factors. Often, reliability is measured using the ICC, which may falsely inflate the reliability estimate.[8,9] Rather, reliability should be based on levels of change within subjects and not within groups of subjects.[10] One way to improve reliability may be repeated measurements, although one should mind the increasing number of missing values. Controlling for secondary influences, such as diet, or monthly cycle, may be advantageous though practically impossible.

Following up on the findings in **chapter 4**, future studies should investigate the regulation and function of NTRK3 in the brain. In order to bring us closer to the identification of causative mutations, a next step may be deep candidate gene sequencing of the tails of population (endo)phenotype distributions to identify a causal variant. Such analysis requires the availability of enormous amounts of population data. Large cohort studies measuring a wide range of phenotypes and environmental factors may enable one to select the traits and factors of interest and test the extremes of the distribution.

As mentioned above, a challenge to the field is the investigation of complicated phenotypic interactions. One way to estimate the proportion of the genetic effects of schizophrenia genes on the endophenotype is to include schizophrenia diagnosis as a covariate in heritability or linkage analysis. Such analysis would reveal whether the genetic effects are overlapping or specific for that endophenotype. The other way around, including an endophenotypic measure as a covariate when analysing schizophrenia would enable one to estimate the proportion of specific and overlapping genetic effects on schizophrenia. Similarly, gene-gene interactions and gene-environment interactions may be dissected if one knows which genes or environmental factors to combine. As gene–environment interactions are included in heritability estimates there may be considerable yet to explore genetic-environmental effects on the development of schizophrenia and endophenotypes. Given that a combination of genes or environmental factors may have large effects on traits, while either has only limited effect,[11] testing of interactions or epistasis will depend on a thorough understanding of biological pathways involved.

Clearly, an integrative approach is necessary for making progress in the field of complex diseases.[7,12] Efforts should be made to combine the best approaches from medicine, cognitive psychology, and genetics; excellent phenotyping combined with clever methodology, high-throughput genetic and functional genomic data, and bioinformatics, in close contact with the fields of proteomics, neuroscience and animal research.

As generally recognised, large study samples are needed to capture the genes with the small effect sizes that are apparent to influence schizophrenia and related endophenotypes. However, larger samples may actually increase genetic and phenotypic heterogeneity and thereby reduce power. Such increase in heterogeneity may be one of the main reasons for recent disappointing results of such enormous datasets. Clearly, besides large study samples combined with advanced technological methods, the only way to accomplish improvements in the field of psychiatric genetics is through an excellent level of phenotyping, including the use of endophenotypes. The findings described in this thesis may serve as a step towards mapping of the Human Phenome,[13,14] for “developing a phenotype ontology or lexicon is a major undertaking, but is necessary in psychiatric genetics to make progress in connecting the genome to the phenome”.

If endophenotypes can facilitate the identification and distinction of genes and pathways involved in several psychiatric disorders, such knowledge may stimulate the development of biologically-based diagnoses and may elucidate environmental risk factors. If vulnerability can be estimated more precisely, secondary risk factors may be avoided. Ultimately, these efforts may accomplish personalised treatment options, early detection, and prevention of psychiatric disorders such as schizophrenia, a chronic and severe brain disease.

References

1. Green AE, Munafo MR, DeYoung CG, Fossella JA, Fan J, Gray JR (2008): Using genetic data in cognitive neuroscience: from growing pains to genuine insights. *Nat Rev Neurosci* 9: 710-720.
2. Meyer-Lindenberg A, Weinberger DR (2006): Intermediate phenotypes and genetic mechanisms of psychiatric disorders. *Nat Rev Neurosci* 7: 818-827.
3. Almasy L, Gur RC, Haack K, Cole SA, Calkins ME, Peralta JM et al (2008): A genome screen for quantitative trait loci influencing schizophrenia and neurocognitive phenotypes. *Am J Psychiatry* 165: 1185-1192.
4. Paunio T, Tuulio-Henriksson A, Hiekkalinna T, Perola M, Varilo T, Partonen T et al (2004): Search for cognitive trait components of schizophrenia reveals a locus for verbal learning and memory on 4q and for visual working memory on 2q. *Hum Mol Genet* 13: 1693-1702.
5. Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I et al (2003): Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 73: 34-48.
6. Ng MY, Levinson DF, Faraone SV, Suarez BK, DeLisi LE, Arinami T et al (2008): Meta-analysis of 32 genome-wide linkage studies of schizophrenia. *Mol Psychiatry* 14: 774-85.
7. Ioannidis JP, Thomas G, Daly MJ (2009): Validating, augmenting and refining genome-wide association signals. *Nat Rev Genet* 10: 318-329.
8. Atkinson G, Nevill AM (1998): Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Med* 26: 217-238.
9. Hopkins WG (2000): Measures of reliability in sports medicine and science. *Sports Med* 30: 1-15.
10. Zandbelt BB, Gladwin TE, Raemaekers M, van BM, Neggers SF, Kahn RS et al (2008): Within-subject variation in BOLD-fMRI signal changes across repeated measurements: quantification and implications for sample size. *Neuroimage* 42: 196-206.
11. Tan HY, Chen Q, Sust S, Buckholtz JW, Meyers JD, Egan MF et al (2007): Epistasis between catechol-O-methyltransferase and type II metabotropic glutamate receptor 3 genes on working memory brain function. *Proc Natl Acad Sci U S A* 104: 12536-12541.
12. Horvath S, Mirnics K (2009): Breaking the gene barrier in schizophrenia. *Nat Med* 15: 488-490.
13. Freimer N, Sabatti C (2003): The human phenome project. *Nat Genet* 34: 15-21.
14. Sabb FW, Burggren AC, Higier RG, Fox J, He J, Parker DS et al (2009): Challenges in phenotype definition in the whole-genome era: multivariate models of memory and intelligence. *Neuroscience doi:* 10.1016/j.neuroscience.2009.05.013.

Nederlandse samenvatting

Dit proefschrift beschrijft een aantal onderzoeken waarin de zoektocht naar erfelijke cognitieve endofenotypen voor schizofrenie en de toepassing van endofenotypen in genetisch onderzoek centraal staat.

Hoofdstuk 1 - Inleiding

Schizofrenie is een complexe en chronische psychiatrische stoornis die bij ongeveer 1% van de bevolking voorkomt.[1,2] De ziekte schizofrenie wordt gekenmerkt door hallucinaties en wanen, zogenaamde positieve symptomen, en verlies van initiatief en interesse en affectvervlakking, zogenaamde negatieve symptomen. Hoewel schizofrenie sterk erfelijk bepaald is (~80%), wordt genetisch onderzoek ernaar bemoeilijkt door het grote aantal genen dat een rol speelt bij schizofrenie en door de kleine effecten van die genen. Ook spelen omgevingsfactoren een rol en de interacties tussen genen onderling en met de omgeving. Er is een lange lijst van kandidaat-genen voor schizofrenie ontstaan in de loop der jaren, maar van weinig genen is tot nu toe overtuigend aangetoond dat ze bij de ontwikkeling van schizofrenie betrokken zijn.
Eén van de belangrijkste belemmeringen bij genetisch onderzoek naar schizofrenie is dat de ziekte een heterogene aandoening is. De diagnose schizofrenie is weliswaar klinisch relevant, maar minder geschikt voor biologisch en genetisch onderzoek. In dit onderzoek is gebruik gemaakt van een nieuwe methode, namelijk het meten van erfelijke, aan schizofrenie gerelateerde kenmerken, de zogenaamde endofenotypen.

Endofenotypen zijn meetbare, aan schizofrenie gerelateerde kenmerken die gemeten kunnen worden in gezonde familieleden van patiënten met schizofrenie. Een broer of een zus van een patiënt deelt bijvoorbeeld gemiddeld 50% van de genen met de patiënt en heeft dus 50% kans drager te zijn van risicogenen voor schizofrenie. De kans dat deze broer of zus ook schizofrenie ontwikkelt is echter aanzienlijk kleiner (~9%) omdat het aantal genen dat een rol speelt bij schizofrenie groot is en de effecten van deze genen klein. Daarnaast zijn er ook de genoemde omgevingsinvloeden, en interacties tussen genen onderling en met de omgeving. Risicogenen bij familieleden zouden wel kunnen resulteren in aan schizofrenie gerelateerde kenmerken, de zogenaamde endofenotypen. Zo blijkt bijvoorbeeld dat familieleden van patiënten met schizofrenie gemiddeld slechter scoren op een aandachtstaak dan een groep controle proefpersonen, maar wel beter dan de patiënten. Schizofrenie gaat gepaard met een brede achteruitgang in informatieverwerkingsprocessen, zoals cognitie, emotie, perceptie en motoriek. Veel testen op deze gebieden

laten ook effecten zien bij familieleden en zouden dus mogelijke endofenotypen voor schizofrenie kunnen zijn. Een kandidaat-endofenotype moet aan een aantal criteria voldoen: het moet aan schizofrenie gerelateerd zijn, erfelijk zijn, stabiel zijn, betrouwbaar te meten zijn, aangetast zijn in familieleden van patiënten met schizofrenie, en - idealiter - voorspellend zijn voor het ontwikkelen van schizofrenie.

Endofenotypen, ook wel *tussenliggende fenotypen* genoemd, vormen als het ware een verbinding tussen genen en een ziekte. Als zodanig kunnen ze gebruikt worden in genetisch onderzoek. Hierbij gaat men uit van de veronderstelling dat een deelaspect (endofenotype) van een fenotype (schizofrenie) door een geringer aantal genen wordt beïnvloed dan het fenotype zelf. Effecten van een geringer aantal genen zouden beter te meten moeten zijn dan van een groter aantal. Op basis van deze redenering zouden in genetisch onderzoek endofenotypen krachtiger kunnen zijn (met een hoger onderscheidingsvermogen) dan de klinische diagnose schizofrenie. Andere voordelen zijn: dat endofenotypen kwantitatieve maten zijn die gemeten kunnen worden in gezonde familieleden en toegepast kunnen worden in meer krachtige kwantitatieve analyses; dat voor zover de onderliggende biologische mechanismen bij endofenotypen bekend zijn of te onderzoeken zijn, deze informatie het zoeken naar positionele kandidaat-genen kan ondersteunen; dat endofenotypen direct(er) vertaald kunnen worden naar dierstudies.

Hoewel het aantal potentiële endofenotypen in de loop der jaren sterk toegenomen is, is het aantal studies waarin endofenotypen werden toegepast in familiestudies relatief beperkt gebleven. Toch hebben verschillende endofenotypen reeds bewezen nuttig te zijn bij het vinden van nieuwe kandidaat-genen voor schizofrenie,[3] of bij het ontrafelen van de genetisch-biologische mechanismen bij schizofrenie.[4,5]

Het doel van het in dit proefschrift beschreven onderzoek is het leveren van een bijdrage aan het ontrafelen van de genetische eigenschappen van veelbelovende endofenotypen voor schizofrenie en deze toe te passen in genetisch onderzoek. Het eerste deel van dit proefschrift is gericht op het onderzoeken van de genetische eigenschappen van endofenotypen voor schizofrenie. Het tweede deel is gericht op de toepassing van endofenotypen in een genoomwijd koppelingsonderzoek. Daarin werd ook gezocht naar positionele kandidaat-genen voor schizofrenie (kandidaat op basis van hun positie op het genoom), die vervolgens werden vergeleken tussen schizofreniepatiënten en een controle groep met behulp van een associatieanalyse.

Kort samengevat zijn de bevindingen van dit onderzoek als volgt: slechts 5 van de 13 onderzochte kandidaat-endofenotypen bezitten een redelijke mate van erfelijkheid (hoofdstuk 2); sommigen van de erfelijke endofenotypen worden door dezelfde genen beïnvloed (genetische correlatie) en andere juist niet (hoofdstuk 3); verschillende chromosoomgebieden lijken gekoppeld te

zijn aan de erfelijke endofenotypen (hoofdstuk 4); verschillende potentiële kandidaat-genen voor schizofrenie zijn gelegen binnen deze gebieden (hoofdstuk 4); het NTRK3 gen lijkt betrokken te zijn bij schizofrenie (hoofdstuk 4); oscillerende hersenactiviteit (EEG) met een lage frequentie gemeten op de achterhoofdskwab (occipitale theta activiteit) is een erfelijk endofenotype (hoofdstuk 5); occipitale theta-activiteit lijkt gekoppeld te zijn aan enkele chromosoomgebieden (hoofdstuk 5).

Hoofdstuk 2

Dit hoofdstuk beschrijft het onderzoek dat zich richtte op het vinden van bruikbare endofenotypen voor genetisch onderzoek bij schizofrenie. Met dit doel werden de erfelijke eigenschappen van 13 kandidaat-endofenotypen voor schizofrenie onderzocht. Het uitgangspunt van deze studie was dat bruikbare endofenotypen voor genetisch onderzoek een patroon van overerving zouden moeten laten zien dat eenvoudiger is dan dat van schizofrenie zelf. Familiare correlaties werden berekend voor de 13 geselecteerde endofenotypen in 25 uitgebreide families waarin schizofrenie vaker voorkomt, gevolgd door erfelijkheid berekeningen en segregatie analyse (het passen van verschillende overervingmodellen). De resultaten toonden aan dat slechts 5 van de 13 endofenotypen matig gecorreleerd zijn tussen familieleden, namelijk prepulse inhibitie, openheid, verbale vaardigheid, vroege visuele perceptie en ruimtelijk werkgeheugen. De erfelijkheid kwam hiermee overeen, variërend van 37% tot 54%. De acht endofenotypen met lage familiaire correlaties bleken de testen te zijn waarvoor eerder onderzoek ook tegenstrijdige of laag erfelijkheid schattingen hadden gerapporteerd. In de segregatie analyse bleek voor twee van de vijf erfelijke endofenotypen, namelijk prepulse inhibitie en openheid, het best passende overervingmodel een eenvoudig model te zijn met een dominant patroon van overerving. Verbale vaardigheid, vroege visuele perceptie en ruimtelijk werkgeheugen konden het best beschreven worden met een poligenetisch of multifactorieel overervingmodel. Samengevat, vooral prepulse inhibitie en openheid lijken veelbelovende kandidaat endofenotypen te zijn voor genetisch onderzoek naar schizofrenie omdat ze een simpeler model van overerving laten zien. Bovendien blijken niet alle kandidaat-endofenotypen erfelijk te zijn in onze onderzoeksgroep. Op grond van de resultaten van dit onderzoek, dient het de aanbeveling om behoedzaam te zijn bij het toepassen van endofenotypen in genetisch onderzoek, temeer omdat menig endofenotype mogelijk niet het voordeel van een eenvoudigere overervingwijze heeft.

Hoofdstuk 3

Het onderzoek in dit hoofdstuk was gericht op het bestuderen van de genetische relaties tussen de vijf endofenotypen waarvan in hoofdstuk 2 was aangetoond dat ze overerfbaar zijn, namelijk, prepulse inhibitie, openheid, verbale vaardigheid, vroege visuele perceptie en ruimtelijk werkgeheugen. Een zesde kenmerk, intelligentie, werd aan deze analyses toegevoegd omdat het een veelbelovend endofenotype voor schizofrenie is dat hoog correleert met een aantal andere endofenotypen, zoals werkgeheugen. Als eerste werden Pearson's correlaties tussen de endofenotypen berekend. Vervolgens werd geschat in hoeverre de gedeelde variantie tussen de endofenotypen verklaard kon worden door genetische of omgevingsfactoren. De resultaten lieten significante correlaties zien tussen ruimtelijk werkgeheugen, verbale vaardigheid en intelligentie en tussen vroege visuele perceptie en ruimtelijke werkgeheugen. De correlatie tussen ruimtelijk werkgeheugen en intelligentie kon voor een groot deel toegeschreven worden aan genetische factoren. In andere woorden, overlappende genetische effecten dragen bij aan individuele verschillen in ruimtelijk werkgeheugen en intelligentie. Overlappende omgevingseffecten werden waargenomen tussen verbale vaardigheid en openheid en tussen verbale vaardigheid en ruimtelijke werkgeheugen. Prepulse inhibitie en openheid bleken relatief afzonderlijke erfelijke entiteiten te zijn; zij delen weinig genetische of omgevings-variantie met verbale vaardigheid, ruimtelijke werkgeheugen, vroege visuele perceptie of intelligentie. Opmerkelijk genoeg zijn prepulse inhibitie en openheid juist de endofenotypen die in hoofdstuk 2 een eenvoudigere wijze van overerving lieten zien. Dit zou betekenen dat deze kenmerken zowel door een geringer aantal als door afzonderlijke genetische factoren beïnvloed worden. Hierdoor zouden deze endofenotypen kunnen bijdragen aan het opsporen van afzonderlijke genetische varianten voor schizofrenie. De bevindingen uit dit onderzoek steunen de hypothese dat meerdere genen worden gedeeld tussen intelligentie, gerelateerde cognitieve eigenschappen en schizofrenie, terwijl een kleiner aantal onafhankelijke genetische factoren bijdragen aan enkele afzonderlijke endofenotypen, zoals prepulse inhibitie en openheid. De prestaties op individuele testen en hun onderliggende factoren lijken dus slechts gedeeltelijk gecorreleerd te zijn. Deze gedeeltelijke correlatie wijst op verschillende aangedane cognitieve dimensies bij schizofrenie, passend bij een poligenetisch model voor schizofrenie. Intelligentie blijkt een veelbelovend endofenotype voor genetisch onderzoek naar schizofrenie te zijn, hoewel het onderliggend genetisch mechanisme waarschijnlijk complex is.

Hoofdstuk 4

In het onderzoek in dit hoofdstuk werden de endofenotypen gekoppeld aan het genotype. In een genoomwijde koppelingsanalyse zochten we naar chromosoomgebieden (loci) waar genen zou-

den kunnen liggen voor de zes endofenotypen waarvan in hoofdstuk 2 en 3 werd aangetoond dat ze erfelijk en deels genetisch gecorreleerd zijn. Hiervoor gebruikten we iets meer dan 6.000 markers (*single nucleotide polymorphisms*, SNPs) verspreid over het genoom. Drie gebieden werden gevonden in de *meerdere-punts* analyse, namelijk 8q21-24 voor vroege visuele perceptie en 16q21-22 en 17p13 voor openheid. In de *twee-punts* analyse waren er aanwijzingen voor verschillende gebieden die gekoppeld zijn aan prepulse inhibitie, verbale vaardigheid, ruimtelijk werkgeheugen en intelligentie, bijvoorbeeld op 2q22.1, 2q37.1, 5q33-34, 9q31-33, en 15q26.1. De meeste van deze gebieden overlappen of liggen dichtbij eerder gevonden loci voor schizofrenie, gerelateerde endofenotypen en andere psychiatrische aandoeningen, zoals bipolaire stoornis. Uit de veronderstelling dat endofenotypen genetische factoren delen met schizofrenie volgt dat er genen voor schizofrenie moeten liggen in (sommige van) de loci voor endofenotypen. Uitgaande van deze veronderstelling werd gezocht naar positionele kandidaat-genen voor schizofrenie en het endofenotype met behulp van prioritering analyse op basis van de functionele verbanden tussen de genen die zijn gelegen in de loci en op basis van biologische mechanismen van bekende kandidaat-genen voor schizofrenie. Voor de geprioriteerde genen werd onderzocht of deze in minder of meerdere mate tot expressie komen in post-mortem hersenweefsel van schizofrenie patiënten vergeleken met controle personen op basis van eerder gepubliceerde en online datasets. Vier geprioriteerde genen lieten een verschil in expressie zien bij schizofrenie. Voor twee van deze genen, namelijk EIF4E2, en vooral NTRK3, vonden we vervolgens een associatie met schizofrenie in een steekproef van 758 patiënten en 676 controle personen. Om de associatie tussen NTRK3 en schizofrenie te toetsen werden dezelfde analyses nog een keer uitgevoerd in twee grotere onafhankelijke patiënt-controle datasets (1.172/1.378 en 921/954 patiënten/controle proefpersonen). In beide replicatiesets waren verschillende markers binnen NTRK3 ook significant geassocieerd met schizofrenie, zelfs sterker, wat de waarschijnlijkheid dat dit gen betrokken is bij schizofrenie verder vergroot. Deze studie laat zien dat endofenotypen, net als schizofrenie zelf, complexe entiteiten zijn waarvoor grote datasets van informatieve en uitgebreide families nodig zijn om koppeling met chromosoomgebieden aan te tonen. Desondanks resulteerde dit onderzoek in het vinden van een potentieel kandidaat-gen voor schizofrenie (NTRK3) door het combineren van de klassieke methode van *positioneel klonen* (koppeling gevold door associatie) met het gebruik van zorgvuldig geselecteerde endofenotypen, kennis over functionaliteit van kandidaat-genen en genexpressie datasets. NTRK3 is een gen dat van belang is bij de ontwikkeling van de hersenen en vormt een nieuw aanknopingspunt voor onderzoek naar het ontstaan van schizofrenie.

Hoofdstuk 5

In het onderzoek in hoofdstuk 5 werd onderzocht of oscillerende hersenactiviteit (electroencephalogram; EEG) een bruikbaar endofenotype is voor toepassing in genoomwijd koppelingsonderzoek. EEG is afwijkend bij patiënten met schizofrenie en voldoet aan de meeste criteria voor een kandidaat-endofenotype. EEG zou een meer nauwkeurige afspiegeling kunnen zijn van de onderliggende genetische effecten dan gedragsmaten (zoals bovengenoemde endofenotypen), zoals eerder gesuggereerd voor andere hersenactiviteit fenotypen.[6,7] In dit onderzoek werd de hersenactiviteit gemeten tijdens de rust intervallen van de P50 taak. Theta-frequenties gemeten op drie elektroden op de achterhoofdskwab (occipitale gebieden) lieten familiaire correlaties zien variërend van 0.18 tot 0.28 met overeenkomstige erfelijkheid schattingen (31-55%). In de segregatie analyse paste dit endofenotype het beste op een Mendeliaans transmissie model, wat overeenkomt met een meer simpele vorm van overerving. Op basis van deze erfelijkheidsschattingen werd occipitale theta-activiteit geselecteerd voor toepassing in genetisch onderzoek. In de koppelingsanalyse vonden we aanwijzingen voor 13 loci om gekoppeld te zijn aan occipitale-theta-activiteit met een empirische waarschijnlijkheid lager dan 0,001: 3p12-11, 3q29, 5p15, 6p22, 6q14-16, 7p22, 11p14, 15q26, 16p13, 19p13, 19p13, 20p13, en 20q13. Zoals ook het geval was bij cognitieve endofenotypen, zijn enkele van deze gebieden eerder in verband gebracht met schizofrenie. Eén van de gekoppelde markers was gelegen binnen het DTNBP1 gen. DTNBP1 is een bekend kandidaat-gen voor schizofrenie, dat ook betrokken is bij cognitief functioneren. Deze bevindingen tonen aan dat theta-activiteit gemeten op occipitale hersengebieden het meest geschikte hersenactiviteit-endofenotype is als het gaat om erfelijke eigenschappen. Hoewel het onderscheidingsvermogen van de koppelingsanalyse laag was, lukte het om potentiële loci voor theta-activiteit aan te wijzen, waaronder een sterk kandidaat-gen voor schizofrenie (DTNBP1). Hersenactiviteit als endofenotype presteerde niet beter dan de cognitieve endofenotypen die in dezelfde families zijn gemeten (hoofdstukken 2 en 3). Kennelijk is theta-activiteit niet een meer nauwkeurige afspiegeling van de onderliggende genetische effecten dan cognitieve gedragsmaten.

Hoofdstuk 6

Dit hoofdstuk beschrijft, na een samenvatting van eerdere hoofdstukken, de methodologische overwegingen, discussie en perspectieven.

De belangrijkste conclusie uit het onderzoek dat in dit proefschrift is beschreven, is dat endofenotypen, mits goed geselecteerd op criteria waaronder erfelijkheid, een bijdrage kunnen leveren aan genetisch onderzoek naar schizofrenie. Verder blijkt een gen dat van belang is bij de ontwik-

keling van de hersenen, NTRK3, een rol te spelen bij de ontwikkeling van schizofrenie en werkgeheugen. Deze bevinding vormt een nieuw aanknopingspunt in de zoektocht naar genen en biologische mechanismen voor schizofrenie.

Een methodologische overweging is dat de beschreven bevindingen gerepliceerd moeten worden in andere populaties. Erfelijkheid schattingen, segregatie modellen en koppelingsanalyses zijn sterk afhankelijk van de bestudeerde populatie, in dit geval uitgebreide Nederlandse families waarin vaker schizofrenie voorkomt. Een ander punt van aandacht is de beperkte steekproefomvang. Niettemin zijn in alle studies verschillende methoden gecombineerd die een consistent patroon van resultaten lieten zien in overeenstemming met theoretische modellen en eerdere bevindingen. Verder is een belangrijke veronderstelling in dit onderzoek geweest dat endofenotypen genetisch gerelateerd zijn aan schizofrenie. Deze relatie hebben wij niet zelf onderzocht, maar wordt ondersteund door eerder onderzoek (hoofdstuk 1).

Op basis van de beschreven bevindingen verdient het aanbeveling om verder onderzoek te doen naar de functie van het NTRK3 gen. Dit gen kan inzicht verlenen in de onderliggende mechanismen die een rol spelen bij schizofrenie en werkgeheugen. Ook verdient het aanbeveling om familieonderzoek als een belangrijk onderdeel van de psychiatrische genetica te behouden. Endofenotypen kunnen juist hierin een belangrijke rol spelen vanwege de mogelijkheid om familieleden goed te beschrijven.

Om een verdere succesvolle toepassing van endofenotypen in genetisch onderzoek te bevorderen is het van belang de eigenschappen van kandidaat-endofenotypen nauwkeuriger te onderzoeken. Uit het onderzoek beschreven in hoofdstuk 3 blijkt een dynamisch patroon van correlaties te bestaan tussen de endofenotypen. Dit patroon is moeilijk maar wel noodzakelijk om te ontrafelen wanneer men meer te weten wil komen over onderliggende specifieke en gedeelde genetische effecten. Endofenotypen zijn vaak niet specifiek voor één psychiatrische stoornis. Het is daarom van belang over de grenzen van verschillende psychiatrische ziektebeelden heen te kijken en de onderliggende verbindende biologische mechanismen op te sporen. Een andere belangrijke kwestie die aandacht verdient is de betrouwbaarheid van endofenotypen. Als de betrouwbaarheid lager is dan 80%, wat gebruikelijk is bij cognitieve taken, kan de optredende meetfout het genetische effect waarin men geïnteresseerd is volledig tenietdoen. Vandaar dat vooral naar endofenotypen met een zeer hoge betrouwbaarheid gezocht moet worden om het onderscheiden van de kleine genetische en omgevingseffecten mogelijk te maken.

De laatste jaren zijn de mogelijkheden binnen genetisch onderzoek spectaculair toegenomen. Dankzij de inspanning van velen zijn de huidige datasets exponentieel in grootte toegenomen met een nog groter aantal beschikbare markers. Deze aantallen, die nodig zijn voor het meten van de kleine genetische effecten waren tot voor enkele jaren nog ondenkbaar. Juist bij deze gi-

gantische datasets blijft het van belang zorg te dragen voor een uitstekende fenotypering. Een kleine meetfout kan een klein effect al snel teniet doen. Een veelbelovend project is het bundelen van krachten voor het ontwikkelen van het 'Humaan Fenoom'; een gedetailleerde beschrijving van een fenotype ontologie als essentiële verbinding tussen DNA en ziekteproces. Hopelijk zullen de beschreven bevindingen en aanbevelingen van dit onderzoek bijdragen tot verbeterde kennis over de onderliggende biologische mechanismen, en uiteindelijk leiden tot verbetering van behandeling, vroege opsporing en preventie van psychische stoornissen zoals schizofrenie, een chronische en ernstige hersenziekte.

Referenties

1. Bhugra D (2005): The global prevalence of schizophrenia. *PLoS Med* 2: e151.
2. Esslinger C, Walter H, Kirsch P, Erk S, Schnell K, Arnold C et al (2009): Neural mechanisms of a genome-wide supported psychosis variant. *Science* 324: 605.
3. Freedman R, Coon H, Myles-Worsley M, Orr-Urtreger A, Olincy A, Davis A et al (1997): Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proc Natl Acad Sci U S A* 94: 587-592.
4. Goldner EM, Hsu L, Waraich P, Somers JM (2002): Prevalence and incidence studies of schizophrenic disorders: a systematic review of the literature. *Can J Psychiatry* 47: 833-843.
5. Green AE, Munafo MR, DeYoung CG, Fossella JA, Fan J, Gray JR (2008): Using genetic data in cognitive neuroscience: from growing pains to genuine insights. *Nat Rev Neurosci* 9: 710-720.
6. Huffaker SJ, Chen J, Nicodemus KK, Sambataro F, Yang F, Mattay V et al (2009): A primate-specific, brain isoform of KCNH2 affects cortical physiology, cognition, neuronal repolarization and risk of schizophrenia. *Nat Med* 15: 509-518.
7. Meyer-Lindenberg A, Weinberger DR (2006): Intermediate phenotypes and genetic mechanisms of psychiatric disorders. *Nat Rev Neurosci* 7: 818-827.

List of Publications

Aukes MF, Alizadeh BZ, Kemner C, van 't Slot R, Strengman E, Sitskoorn MM, Sinke, RJ, Kahn RS, Ophoff RA. (2009). A Genome-wide Linkage Study of Cognitive Endophenotypes for Schizophrenia highlights the Neurotrophin receptor gene (NTRK3) as a Susceptibility Locus. Submitted

Aukes MF, Boks, MPM, Willems AE, van Lutterveld R, Sommer IE, Sitskoorn MM, Ophoff RA, Alizadeh BZ, Kahn RS. (2009). A Genome-wide Linkage Scan of Theta Band Activity as a Heritable Endophenotype for Schizophrenia. Submitted.

Aukes MF, Alizadeh BZ, Sitskoorn MM, Kemner C, Ophoff RA, Kahn RS. (2009). Genetic Overlap Among Intelligence and Other Candidate Endophenotypes for Schizophrenia. *Biological Psychiatry*, 65: 527-534.

Aukes MF, Alizadeh BZ, Sitskoorn MM, Selten J-P, Sinke RS, Kemner C, Ophoff RA, Kahn RS (2008). Finding suitable phenotypes for molecular genetic studies of schizophrenia. *Biological Psychiatry*, 64: 128-136.

Muntjewerff JW, Hoogendoorn ML, **Aukes MF**, Kahn RS, Sinke RJ, Blom HJ, Heijer MD. (2007). No evidence for a preferential transmission of the methylenetetrahydrofolate reductase 677T allele in families with schizophrenia offspring. *American Journal of Medical Genetics part B – Neuropsychiatric Genetics*, 144B: 891-894.

Ito J, Nikolaev A, Luman M, **Aukes MF**, Nakatani C, van Leeuwen CC (2003). Perceptual switching, eye-movements, and the bus-paradox. *Perception*. 32, 681-698.

Conference Abstracts

Aukes MF, Alizadeh BZ, Sitskoorn MM, Kemner C, van 't Slot R, Koeleman BPC, Ophoff RA, Kahn RS. (2008). A Genome Wide Linkage Study of Endophenotypes for Schizophrenia in Extended Families. WCPG Osaka, Japan. **(Oral)**

Aukes MF, Alizadeh BZ, Sitskoorn MM, Selten J-P, Kemner C, Ophoff RA, Kahn RS. (2008). Shared Genetics of Candidate Endophenotypes for Schizophrenia: Multivariate Heritability Analysis. *Schizophrenia Research*, 102/1-3 (Supplement 2), 369. **(Poster)**

Aukes MF, Alizadeh BZ, Sitskoorn MM, Kemner C, Selten J-P, Ophoff RA, Kahn RS. (2007). Pattern of inheritance of candidate endophenotypes for schizophrenia: a segregation analysis. WCPG, NY, USA, http://www.wcpg2007.com/programs.html. **(Oral)**

Aukes MF, Alizadeh BZ, Selten J-P, Sinke RJ, Sitskoorn MM, Kahn RS. (2007). Genetics of schizophrenia: Segregation analysis of endophenotypes. *Schizophrenia Bulletin*, 33 (2), 275. **(Poster)**

Aukes MF, Alizadeh BZ, Sitskoorn MM, Selten J-P, Sinke RJ, Kahn RS. (2006). Genetics of schizophrenia: Segregation analysis of endophenotypes. *American Journal of Medical Genetics part B – Neuropsychiatric Genetics*, 141B (7), 776. **(Poster)**

Aukes MF, Selten J-P, Sinke RJ, Sitskoorn MM, Alizadeh BZ, Kahn RS. (2006). Segregation analysis of spatial span: a candidate endophenotype for schizophrenia. *Schizophrenia Research*, 81 (Supplement), 215. **(Poster)**

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About the author

Maartje Aukes was born on January 13th 1976 in Groningen. In 1994, she finished secondary school at the Praedinius Gymnasium in Groningen. Afterwards, she took courses in English and Philosophy at the University of East Anglia, Norwich, United Kingdom. In 2001, she obtained her Master's degree in Psychology with honour at the Psychonomics department of the University of Amsterdam. Her final research project on integration processes in visual information processing was performed at the University of Sunderland, Sunderland, United Kingdom. Afterwards, she continued research on visual perception under supervision of Prof.dr. C.C. van Leeuwen at the Laboratory for Perceptual Dynamics of the RIKEN Brain Science Institute, Saitama, Japan. In September 2002 she started the PhD-project as described in this thesis under supervision of Prof.dr. R.S. Kahn and Prof.dr. M.M. Sitskoorn at the Rudolf Magnus Institute of Neurosciences, Department of Psychatry, University Medical Center Utrecht (UMCU). Currently, she takes a postdoctoral position in a collaboration between the Julius Center for Health Sciences and Primary Care and Department of Psychiatry, at the UMCU.

Maartje Aukes werd op 13 januari 1976 geboren te Groningen. Zij behaalde haar Gymnasium diploma in 1994 op het Praedinius Gymnasium te Groningen. Vervolgens studeerde zij een jaar Engels en Filosofie aan de University of East Anglia te Norwich, Engeland. In 2001 studeerde zij af voor de studie Psychologie bij de vakgroep Psychonomie aan de Universiteit van Amsterdam. Haar afstudeeronderzoek over integratie processen bij visuele informatie verwerking voerde zij uit aan de University of Sunderland te Sunderland, Engeland. Daarna zette zij haar onderzoek naar visuele perceptie onder leiding van Prof.dr. C.C. van Leeuwen voort in het Laboratory for Perceptual Dynamics van het RIKEN Brain Science Institute te Saitama, Japan. In 2002 begon zij aan haar promotieonderzoek (zoals beschreven in dit proefschrift) onder leiding van Prof.dr. R.S. Kahn en Prof.dr. M.M. Sitskoorn aan het Rudolf Magnus Instituut voor Neurowetenschappen, afdeling Psychiatrie, Universitair Medisch Centrum Utrecht (UMCU). Per 1 juli 2009 is zij werkzaam als postdoc in een samenwerkingsverband tussen het Julius Centrum en de afdeling Psychiatrie van het UMCU.