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Genome-wide scan of homogeneous subtypes of NIMH genetics initiative schizophrenia families

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Abstract

In the light of the potential etiological heterogeneity of schizophrenia, we reanalyzed the NIMH genetics initiative data for schizophrenia. We performed linkage analyses on schizophrenia families divided into more homogeneous subgroups. The African-American and European-American families were divided into groups that were successively more homogeneous. The first group included schizophrenia families that were highly familial, meaning that they contained a minimum specified number of affected individuals. We also excluded patients with environmental influences that may affect disease status. These influences included obstetric complications (OC) and viral infections during the neurodevelopmental stage (VIN). In the African-American sample, a linkage analysis of highly familial schizophrenia families without any environmental influences resulted in a single-point LOD (SLOD) score of 2.90, a multipoint LOD (MLOD) of 2.11, a single-point heterogeneity LOD (SHLOD) score of 3.04, and a multipoint heterogeneity LOD (MHLOD) score of 2.11 at marker D8S1819 (8p23.1) under a dominant parametric model. The highly familial European-American schizophrenia families resulted in an SLOD of 0.91 and an MLOD of 1.85, an SHLOD of 1.64 and an MHLOD of 1.97 at marker D22S1169 (22q13.32) using a recessive parametric model. Although this work should be interpreted cautiously and requires replication, these results suggest that schizophrenia may be linked to chromosomal regions 8p23.1 and 22q12.3–q13.32.

Keywords: Schizophrenia; Highly familial schizophrenia; Genome-wide linkage analysis; D8S1819; D22S1169

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1. Introduction

Many researchers have reported positive linkage and association findings with schizophrenia. Although some of these studies exceeded the threshold for statistical significance, initial findings were often not replicated (Riley and McGuffin, 2000; Baron, 2001). One of the most important causes of these conflicting results may be the potential etiological heterogeneity of schizophrenia. Some investigators have suggested that schizophrenia is not a single disease entity but may reflect common symptomatology caused by many genetic and environmental factors (Tsuang et al., 1990; Tsuang and Faraone, 1995; Tsuang, 2000; Sawa and Snyder, 2002).

In the light of strategies used for other non-Mendelian disorders, the genetic complexity of schizophrenia may be simplified by focusing on rare inherited forms of the disorder. Alzheimer's disease provides one example where a series of studies targeted subtypes of the disease that led to successful gene identification (St. George-Hyslop et al., 1987; Pericak-Vance et al., 1991; Schellenberg et al., 1992; Levy-Lahad et al., 1995; Sawa and Snyder, 2002). First Goate et al. (1991) found a rare early-onset form of Alzheimer's disease that had an autosomal dominant form of transmission. It was then found that this form of transmission was caused by a mutation in a single major gene, amyloid precursor protein gene. Later, the association of apolipoprotein E gene with Alzheimer's disease was identified by looking at common, late onset, and sporadic patients (Corder et al., 1993). Diabetes mellitus is another disease that is better understood as a result of studies focusing on disease subtypes. Insulin-dependent diabetes mellitus and the more common non-insulin-dependent diabetes mellitus have been studied as distinct forms. Successful gene identification, including the NIDDM1 (calpain-10 gene) and the NIDDM2 loci, has also been found with non-insulin-dependent diabetes mellitus through the use of subgroups. Furthermore, maturity-onset diabetes of the young (MODY) has been studied as an independent form of the disorder (Vionnet et al., 1992; Davies et al., 1994; Hashimoto et al., 1994; Mahtani et al., 1996; Hanis et al., 1996; Yamagata et al., 1996a, 1996b; Stoffers et al., 1997; Concannon et al., 1998; Horikawa et al., 2000). Breast cancer is a third example of a disease in which researchers have had success using specific subsets of the cases. Hall et al. (1990) found that heterogeneity of linkage was associated with age at onset of disease in familial breast cancer pedigrees. They found that a rare early-onset form of breast cancer was linked to chromosome 17q21. This finding was then replicated by Narod et al. (1991) and, subsequently, Miki et al. (1994) identified BRCA1 as a susceptibility gene for breast and ovarian cancer.

Evidence from in other diseases also suggests that one should focus on reducing etiological heterogeneity in genetic studies of schizophrenia. If the genetic etiology of schizophrenia is heterogeneous, then it is plausible that use of more homogeneous subsets may be a better strategy for gene identification. In this study, homogeneous sub-groupings were determined by (1) classifying families according to the presence or absence of environmental exposures known to influence schizophrenia and (2) identifying families that seem to have a stronger genetic component (those families with more than two affected individuals). Our hypothesis is that those families without identified environmental exposures will form a schizophrenia grouping whose causal pathway to disease is more likely to be affected by genes and not the environment. Furthermore, we propose that families with many affected members are also likely to have a strong genetic loading for schizophrenia (we denote this group as 'highly familial'). Therefore, we reanalyzed data from the National Institute of Mental Health (NIMH) genetics initiative for schizophrenia (Cloninger et al., 1998; Faraone et al., 1998; Kaufmann et al., 1998) by removing all families with environmental exposures in this study and dividing the sample into subgroups based on ethnicity, the number of affected individuals in each family, and the pedigree.

2. Methods

2.1. Subjects

The data were separated into two ethnic groups based on previous findings of heterogeneity in this sample (Cloninger et al., 1998). All affected family

members met DSM-III-R (American Psychiatric Association, 1987) criteria for schizophrenia or schizoaffective disorder, depressive type. The African-American sample included 28 pedigrees with 35 nuclear families and 42 genotyped independent affected sib-pairs (96 genotyped subjects; 70 schizophrenic and 8 schizoaffective disorder patients). The European-American sample consisted of 39 pedigrees including 41 nuclear families with 46 genotyped independent affected sib-pairs (133 genotyped subjects; 74 schizophrenic and 14 schizoaffective disorder patients).

Clinical data were collected on this sample using the Diagnostic Interview for Genetic Studies (DIGS) version 2.0 (Nurnberger et al., 1994; Faraone et al., 1996). The structured interview was supplemented with data from medical records and a semi-structured itemized assessment of psychopathology in family members, called the Family Instrument for Genetic Studies (FIGS). Best estimate diagnoses were made by two experienced psychiatrists or psychologists based on all available information.

These families were originally ascertained by cooperative agreements between the NIMH and investigators at Washington University, Harvard University, and Columbia University. A detailed description of the ascertainment and extension rules, diagnostic assessment, and informed consent has been presented in previous publications (Cloninger et al., 1998; Faraone et al., 1998; Kaufmann et al., 1998).

Highly familial schizophrenia pedigrees were defined as those pedigrees having at least one nuclear family with either (1) schizophrenia or schizoaffective disorder in at least one parent of the affected children or (2) at least three siblings with schizophrenia or schizoaffective disorder. Families that did not meet these criteria composed the less densely affected subgroup.

To further generate homogeneity in the highly familial schizophrenia sample, we excluded possible cases of schizophrenia possibly caused by non-genetic factors. Research suggests that obstetric complications (OC) and viral infections during neurodevelopment (VIN) can lead to schizophrenia (Tsuang and Faraone, 1995; Tsuang, 2000). Therefore, we excluded schizophrenic or schizoaffective disorder patients with either of these conditions from the highly familial schizophrenia sample using information provided in the DIGS. The DIGS asks the following questions that were answered in patient interviews: (1) "Was your own birth or early development abnormal in any way?"; (2) "Were there any problems with your mother's health while she was pregnant with you, or with your birth, such as prematurity or birth complications?"; and (3) "Was your development abnormal in any way, for example, did you walk or talk later than other children?" Schizophrenic or schizoaffective disorder patients with a clearly positive answer to at least one of the above questions were defined as having an environmental exposure that may influence schizophrenia and were therefore removed from the highly familial subgroup. If a highly familial schizophrenia family did not fulfill the criteria for "highly familial" after the removal of these individuals, this family was excluded from the highly familial grouping. There are many drawbacks to using the DIGS to determine the OC or VIN status of individuals. One disadvantage of this strategy is that in most cases it is the patient and not the mother of the affected individual who is responding to the question. Receiving information about early childhood exposures from the patients is less reliable than receiving this information directly from the mother. For this reason it is likely that exposure misclassification of these environmental complications occurred. Although some misclassification is likely, we believe that this procedure will still minimize the number of patients with OC or VIN in our sample, which is the ultimate goal of using these environmental exposure questions. Therefore, although the strategy is not flawless, we believe that it will eliminate a group of individuals with the specified environmental exposures, thereby increasing the homogeneity of the resulting sample.

After exclusion of individuals with the specified environmental exposures, the highly familial African-American sample comprised 16 pedigrees, including 20 nuclear families with 26 independent affected sib-pairs (60 genotyped subjects; 43 schizophrenic and 6 schizoaffective disorder patients). The environmental complications in this sample included premature birth, heart disease, lack of oxygen, and pneumonia. Once environmental cases were removed, the highly familial European-American sample consisted of 9 pedigrees, including 11 nuclear families with 15

independent affected sib-pairs (37 genotyped subjects; 22 schizophrenic and 6 schizoaffective disorder patients). Unfortunately, the removal of these individuals from the sample resulted in too few individuals for genetic analysis.

Through this procedure, we have developed the following schizophrenia subgroups for both ethnicities: (1) the entire ethnic group with no individuals removed; (2) a subgroup that is highly familial, but still includes individuals with possible environmental influences (OC or VIN); (3) a highly familial subgroup without OC and VIN environmental influences. In the African-American sub-sample, we compared the genetic findings of all three groupings. Due to sample size limitations, we only compared the first two grouping in the European-American sample.

2.2. Genotyping

Genotyping details are provided in full detail in the original articles using these data (Cloninger et al., 1998; Faraone et al., 1998; Kaufmann et al., 1998). We describe the procedure briefly. Samples of DNA were extracted from lymphoblastoid cell lines established and maintained by the NIMH Cell Repository at the Coriell Institute for Medical Research. The genotypes of each subject were determined with the Millennium Marker Set (Millennium Pharmaceuticals, Cambridge, MA). This screening set includes 458 DNA microsatellite markers across all chromosomes and spaced at mean intervals of 10 cM. Genotyping was performed at Millennium Pharmaceuticals Markers were amplified by polymerase chain reaction (PCR). Electrophoresis was performed with an ABI

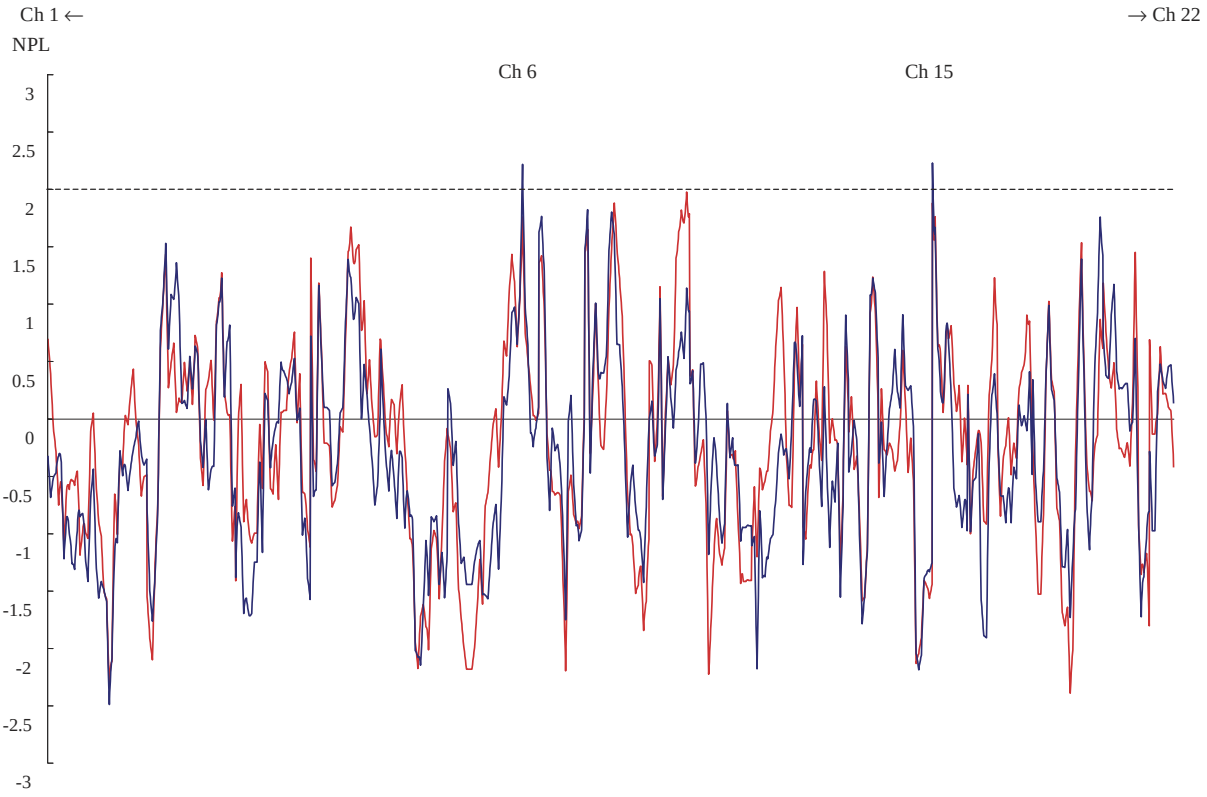

Fig. 1. Genome-wide NPL scores of highly familial schizophrenia and entire schizophrenia in the African-American sample. Red line: entire schizophrenia; Blue line: highly familial schizophrenia; Ch: chromosome. First row shows that left side on the line graph is chromosome 1, and right side is chromosome 22. Second row shows chromosomes including markers with NPL≥2.0 or $P<0.01$ in familial schizophrenia (chromosomes 6 and 15). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PRISM 377 DNA sequencer (Applied Biosystems, Foster City, CA). Alleles were identified with GeneScan 2.0.2 and Genotyper softwares 1.1.1 (Applied Biosystems).

2.3. Statistical analyses

We performed linkage analysis using GENEHUNTER version 2.0 (Kruglyak et al., 1996). Nonparametric multipoint linkage analyses were assessed using nonparametric LOD scores (NPL) and *P*-values. In regions that had the highest NPL scores, we performed single-point (two-point) and multipoint parametric linkage analyses using both dominant and recessive models. Parametric methods were employed because they are known to be more powerful than nonparametric methods under correct or even approximately correct model specification for Mendelian diseases (Durner et al., 1999). Linkage was assessed through the LOD scores throughout the genome while locus heterogeneity was evaluated through the heterogeneity LOD score. In parametric analysis, we used penetrances of 0.65, 0.65 and 0.0096 for the mutant homozygote, mutant heterozygote and wild-type homozygote, respectively, and a mutant allele frequency of 0.005 was employed in the dominant model. Similarly for the recessive model, penetrances were 0.65, 0.0096, and 0.0096 for the mutant homozygote, mutant heterozygote, and wild-type homozygote, respectively, and a mutant allele frequency of 0.11 was used, as suggested by Blouin et al. (1998). Linkage results were interpreted based on

Table 1
NPL and LOD scores of entire schizophrenia (ES), highly familial schizophrenia (HFS) and HFS without OC or VIN in the African-American sample

Marker	NPL (*P*-value) SLOD MLOD	ES: NP=28 NNF=35 NAFM/NNAFM = 85/124 (40.7%[a]) NGIASP=42	HFS: NP=19 NNF=25 NAFM/NNAFM = 68/96 (41.5%[a]) NGIASP=33	HFS without OC or VIN: NP=16 NNF=20 NAFM/NNAFM = 62/85 (42.2%[a]) NGIASP=26
D2S1322–D2S405	NPL	1.44	1.53	2.08*
	(*P* value)	(0.0774)	(0.0669)	(0.0221)
D2S1391	NPL	1.06	1.02	2.09*
	(*P* value)	(0.1462)	(0.1541)	(0.0217)
D6S1009	NPL	1.76	2.22*	1.97
	(*P* value)	(0.0417)	(0.0165)	(0.0282)
D8S1819	NPL	1.65	1.82	2.58*
	(*P* value)	(0.0519)	(0.0385)	(0.0067)
	SLOD			2.90 (SHLOD: 3.04)
	MLOD			2.11 (MHLOD: 2.11)
D8S1791	NPL	1.48	1.80	2.26*
	(*P* value)	(0.0718)	(0.0400)	(0.0145)
D15S128	NPL	1.88	2.23*	1.93
	(*P* value)	(0.0321)	(0.0159)	(0.0303)

NP: number of pedigrees including nuclear families with genotyped sib-pair; NNF: number of nuclear families with genotyped sib-pairs; NAFM/NNAFM: number of affected family members/number of non-affected family members; NGIASP: number of genotyped independent affected sib-pairs.

[a] NAFM/(NAFM+NNAFM). To compare all groups, schizophrenia or schizoaffective disorder with OC or VIN was counted for schizophrenia or schizoaffective disorder in NP, NNF, and NGIASP of whole schizophrenia and familial schizophrenia. In NAFM and NNAFM, the affected family members had schizophrenia, schizoaffective disorder or schizophrenia spectrum disorder. Schizophrenia spectrum disorder included schizotypal personality disorder, non-affective psychotic disorder (schizophreniform disorder, delusional disorder, psychotic disorder not otherwise specified) or mood-incongruent psychotic depressive disorder. We counted genotyped schizophrenic or schizoaffective disorder patients and all of their first-degree relatives. Schizophrenia, schizoaffective disorder, or schizophrenia spectrum disorder with OC or VIN was not counted for schizophrenia, schizoaffective disorder, or schizophrenia spectrum disorder in NAFM/NNAFM of all groups. In NPL, asterisks show NPL≥2 or $P<0.01$.

the recommended significance levels of Lander and Kruglyak (1995).

3. Results

3.1. African-American

Fig. 1 and Table 1 compare the nonparametric linkage analysis findings from of the entire African-American sample with the findings from the highly familial subgroup that includes individuals with environmental influences. While none of the results reached statistical significance, the following regions had NPL scores greater than 2.0 in the highly familial schizophrenia subgroup: D6S1009: 6q23.2 (NPL=2.22, P=0.0165) and D15S128: 15p11.2 (NPL=2.23, P=0.0159).

Fig. 2 and Table 1 show the results of non-parametric linkage analyses from the entire African-American sample and the highly familial grouping that excludes OC and VIN complications. No region showed significant or suggestive evidence for linkage in the highly familial subgroup without environmental complications. In addition, the two peak NPL scores decreased (D6S1009 and D15S128), while the NPL in several other regions was greater than 2.0: D2S1322–D2S405: 2p23.2–p23.3 (NPL=2.08, P=0.0221), D2S1319: 2q33.3 (NPL=2.09, P=0.0217), D8S1819: 8p23.1 (NPL=2.58, P=0.0067), and D8S1791: 8p11.22 (NPL=2.26, P=0.0145). Moreover, the marker with the highest NPL, D8S1819, showed a single-point LOD score (SLOD) of 2.90 and a multi-point LOD score (MLOD) of 2.11 (correct position: 5.96 cM upstream from D8S1819) in the dominant model (Table 1), and an SLOD of 0.14 and an MLOD

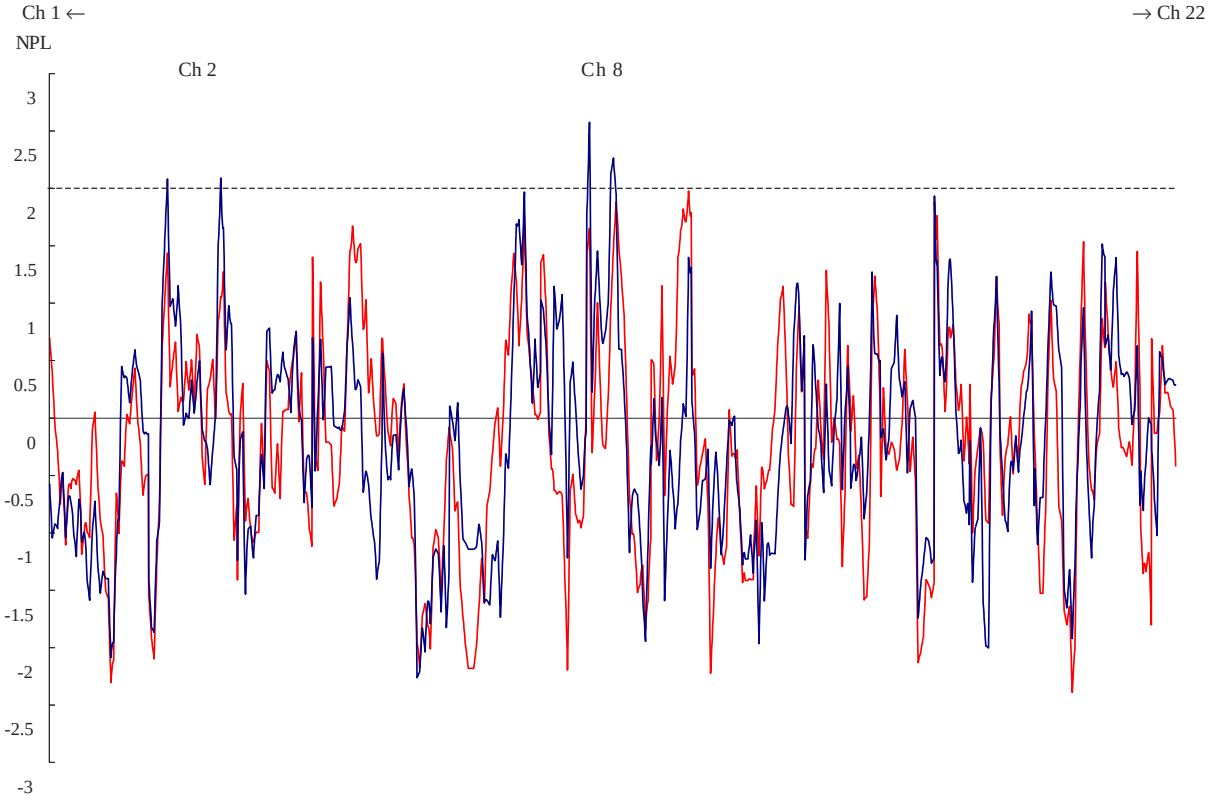

Fig. 2. Genome-wide NPL scores of highly familial schizophrenia without OC or VIN and entire schizophrenia in the African-American sample. Red line: entire schizophrenia; Blue line: highly familial schizophrenia without OC or VIN; Ch: chromosome. Second row shows chromosomes including markers with NPL≥2.0 or P<0.01 in familial schizophrenia without OC or VIN (chromosomes 2 and 8). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of −1.90 in the recessive model. Allowing for locus heterogeneity, D8S1819 showed a single-point heterogeneity LOD (SHLOD) of 3.04 (α=0.88) and a multipoint heterogeneity LOD (MHLOD) of 2.11 (α=0.89; correct position: 3.96 cM upstream from D8S1819) in the dominant model (Table 1). Using the standards established by Lander and Kruglyak (1995), the above NPL scores at D8S1819 did not show significant or suggestive evidence of linkage; however, all of the above LOD scores at this marker did show suggestive evidence of linkage under the dominant model.

3.2. European-American

Fig. 3 and Table 2 show the results of non-parametric linkage analysis for the entire European-American sample and the highly familial schizophrenia subgroup, including individuals with environmental exposures. Neither grouping showed statistically significant evidence for linkage. While there were a two chromosomal regions above 2.0 in the entire European grouping, there were many regions the achieve this threshold in the highly familial sample: D2S411: 2p13.2 (NPL=2.37, *P*=0.013), D2S405: 2q14.2 (NPL=2.05, *P*=0.026), D12S1042: 12p11.23 (NPL=2.34, *P*=0.014), D14S588: 14q24.1 (NPL=2.16, *P*=0.020), and D22S1169: 22q13.32 (NPL=2.41, *P*=0.012; correct position: 7.39 cM downstream from D22S1169).

Moreover, the marker with the highest NPL in the highly familial sample, D22S1169 had a SLOD of 0.91 and a MLOD of 1.85 in the recessive model (Table 2), and a SLOD of 0.87 and a MLOD of 1.02 (correct position: 7.39 cM downstream from D22S1169) in the dominant model. Allowing for

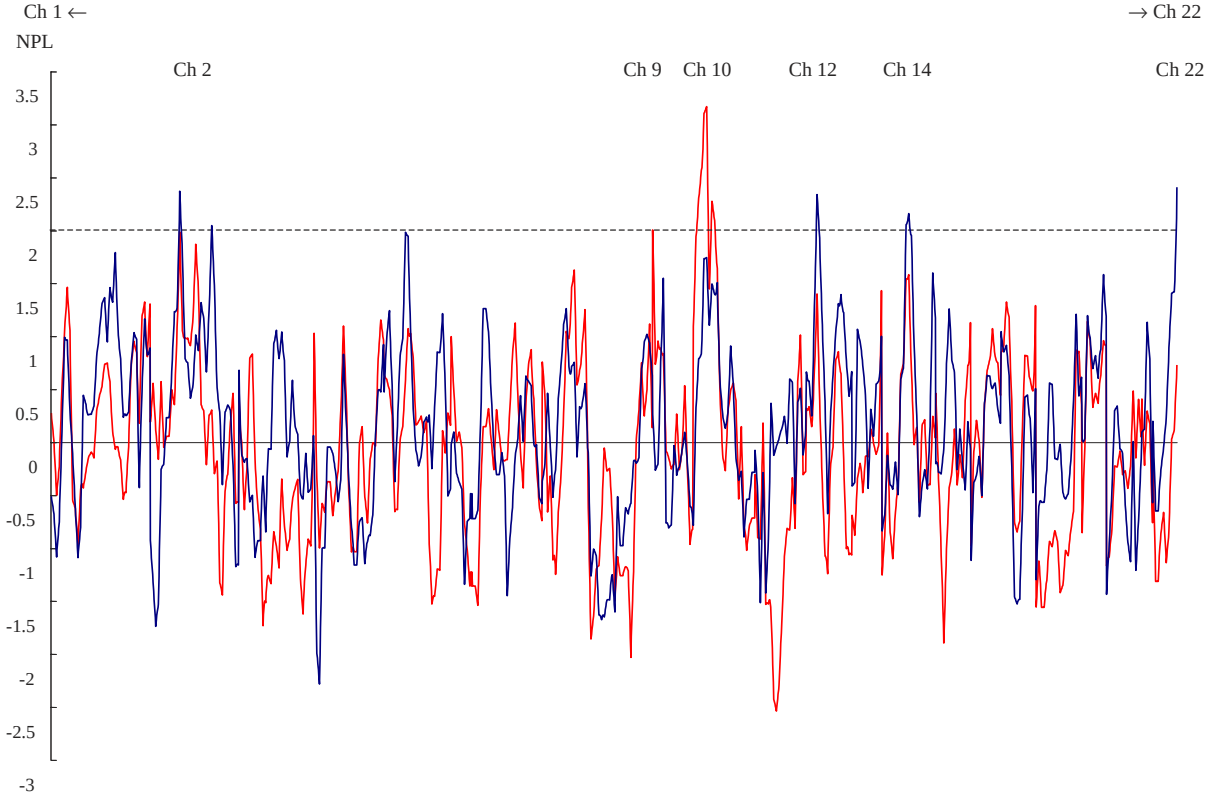

Fig. 3. Genome-wide NPL scores of highly familial schizophrenia and entire schizophrenia in the European-American sample. Red line: entire schizophrenia; Blue line: highly familial schizophrenia; Ch: chromosome. Second row shows chromosomes including markers with NPL≥2.0 or *P*<0.01 in WS (chromosomes 9 and 10) and familial schizophrenia (chromosomes 2, 12, 14, and 22). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 2
NPL and LOD scores of entire schizophrenia (ES) and highly familial schizophrenia (HFS) in the European-American sample

Marker	NPL (*P*-value) SLOD MLOD	ES: NP=39 NNF=41 NAFM/NNAFM = 104/142 (42.3%[a]) NGIASP=46	HFS: NP=12 NNF=14 NAFM/NNAFM = 44/55 (44.4%[a]) NGIASP=19
D2S411	NPL	1.99	2.37*
	(*P* value)	(0.02428)	(0.01277)
D2S1326	NPL	0.31	2.05*
	(*P* value)	(0.37653)	(0.02556)
D9S288	NPL	2.01*	0.27
	(*P* value)	(0.02319)	(0.38269)
D10S582	NPL	3.17*	1.75
	(*P* value)	(0.00088)	(0.04619)
D10S604	NPL	2.28*	1.50
	(*P* value)	(0.01199)	(0.07243)
D12S1042	NPL	1.40	2.34*
	(*P* value)	(0.08120)	(0.01367)
D14S588	NPL	1.59	2.16*
	(*P* value)	(0.05754)	(0.02010)
D22S1169	NPL	0.73	2.41*
	(*P* value)	(0.23098)	(0.01169)
	SLOD		0.91 (SHLOD: 1.64)
	MLOD		1.85 (MHLOD: 1.97)

Schizophrenia or schizoaffective disorder with OC or VIN was counted for schizophrenia or schizoaffective disorder. In NPL, asterisks show NPL≥2 or $P<0.01$.

locus heterogeneity, this marker showed an SHLOD of 1.64 (α=0.61) and a MHLOD of 1.97 (α=0.65; correct position: 7.39 cM downstream from D22S1169) in the recessive model (Table 2). According to Lander and Kruglyak (1995), MHLOD at D22S1169 showed evidence of suggestive linkage.

4. Discussion

In this study we used linkage analysis to compare findings in more and less homogeneous schizophrenia subgroups. For the highly familial African-American subgroup without environmental exposures, an SLOD of 2.90, MLOD of 2.11, SHLOD of 3.04 and MHLOD of 2.11 resulted from the dominant model at D8S1819 (8p23.1). Our method for increasing the homogeneity of the group appears to have worked, as there was no statistical evidence for heterogeneity in our findings (LOD: 2.9 vs. SHLOD: 3.04; MLOD: 2.11 vs. MHLOD: 2.11). The fact that these numbers are so similar provides evidence that our groups are homogenous. For the European-American highly familial subgroup, D22S1169 (22q13.32) showed an SLOD of 0.91, MLOD of 1.85, SHLOD of 1.64 and MHLOD of 1.97 in the recessive model.

From this analysis of the African-American sample, it appears as if LOD scores were higher in general in the highly familial subtype that excluded environment exposures. Therefore, selecting families with clusters of affected individuals and excluding individuals with environmental exposures such as OC or VIN may generate more genetically homogenous subtypes that can detect susceptibility gene(s) for schizophrenia.

The results from the African-American sample suggest that chromosomal region 8p23.1 may be linked to schizophrenia under an autosomal dominant inheritance model. Furthermore, some past studies have implicated this region for linkage to schizophrenia. Using a dominant parametric model, Blouin et al. (1998) found an MLOD of 3.19 and an MHLOD of 4.54 near D8S1771 in a sample of 54 multiplex families. Our marker, D8S1819 maps to chromosomal region 8p23.1 (UniSTS: 64706) which is 6910 kb from a pter of chromosome 8 in the STS map of the NCBI (National Center for Biotechnology Information)/Map Viewer, build 33 (http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi). Marker D8S1771, used in Blouin et al.'s study, is 18,351 kb away from our marker. Moreover, some other previous 8p linkage findings under the dominant model are also close to Blouin et al.'s peak (Kendler et al., 1996; Brzustowicz et al., 1999). However, Kendler et al. (1996) showed positive results at a marker near the Blouin et al. (1998) peak as well as an SHLOD of 2.00 at the marker D8S1731 near our peak. This report also found two distinct peaks (D8S1731: NPL=2.43, P=0.008 and D8S298: NPL=2.51, P=0.006) (Kendler et al., 1996) using nonparametric methods. Another study divided the sample into three subsamples (Straub et al., 2002) and found an SHLOD of 2.20 in one subsample and an MHLOD of 1.52 at D8S1731 in the total sample, which is proximal to the finding presented here (8,103 kb). In contrast to our sample, this sample was largely Caucasian, and a broad diagnosis for schizophrenia was used. In summary, although previous findings have been

reported on chromosome 8p, several of the findings are in a region that is different from the findings here (Kendler et al., 1996; Blouin et al., 1998; Brzustowicz et al., 1999); however, some of the findings from Kendler et al. (1996) and Straub et al. (2002) may support the results presented here.

The results from the highly familial European-American subgroup suggested the possibility that chromosomal region 22q13.32 is linked to schizophrenia under an autosomal recessive model. No report showed a single-point or multipoint LOD greater than three under homogeneity or heterogeneity in chromosome 22. In past studies, two independent samples yielded multipoint LOD scores greater than 1.9, which is deemed suggestive according to Lander and Kruglyak (1995). Blouin et al. (1998) showed a peak recessive MHLOD of 2.10 at D22S1265 (22q12.3) using 54 multiplex families. Coon et al. (1994) reported a recessive MLOD (three-point) of 2.06 near D22S279 (22q13.2) using nine multiplex families. These reported linkages are proximal to our findings. In addition, all of these analyses used a recessive parametric model. The density of affected individuals in our study is similar to the density in past studies with congruent findings. For example, the 54 families used in Blouin et al. (1998) were based on families used in Pulver et al. (1995); 24 of 57 had more than three affected in their families. This uniformity of density in affected individuals could be identifying a subgroup of schizophrenic families who have a stronger genetic loading. Seeing that both studies identify the same chromosomal region, it is also likely that if the findings are not spurious, the families in both studies are genetically similar. Similarly, nine families used in Coon et al. (1994) contained three to six affected. Thus, their sample may also be similar to our familial schizophrenia sample.

It is interesting to note that the percentage of nuclear families with highly familial schizophrenia is higher in the African-American sample (71.4%) than in the European-American sample (34.1%). This further suggests that genetic heterogeneity may exist between these ethnic groups and validates the reports that suggest that these groups should be analyzed separately.

There are several major limitations of this study that should be considered. First, the sample size in each of the subgroups is notably small. This represents a severe limitation of the study that should not be overlooked, as the power for any one study is low. This type of limitation, however, is always going to be a factor whenever a strategy that subdivides a sample is implemented. Secondly, the procedure used to identify individuals with OC and VIN is likely to result in misclassification of this exposure. A third limitation that must be considered is that family size will influence the assignment of a family to a group. For example, if a family has a high genetic loading, but only had two affected offspring, by definition this family will not be placed in the highly familial grouping. This is a notable limitation and there are perhaps better ways to group families; however, one must note that using our definition should make the highly familial group more similar to the overall group. Therefore, losing these families should not bias the findings from the highly familial group but will reduce the overall power to find genetic effects for this grouping Finally, in this analysis, we must consider that we performed multiple full linkage scans. Therefore, when interpreting the LOD score values from these analyses, we must consider that the LOD score for a significant finding is actually higher than what it is in most articles where only one genome scan was performed.

In conclusion, these findings suggest that using more genetically homogeneous schizophrenia families without environmental complications known to cause the disorder may be a more effective way to identify susceptibility loci. In addition, this study builds upon previous findings that implicate chromosomal regions 8p23.1 in an autosomal dominant model and 22q12.3q–q13.32 in an recessive model. These findings should be interpreted cautiously, however, as the sample size is notably small, resulting in low power and there could be misclassification of exposure information.

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References

American Psychiatric Association, 1987. Diagnostic and Statistical Manual of Mental Disorders. (Third Edition, Revised) American Psychiatric Press, Washington, DC.

Baron, M., 2001. Genetics of schizophrenia and the new millennium: progress and pitfalls. American Journal of Human Genetics 68, 299–312.

Blouin, J.L., Dombroski, B.A., Nath, S.K., Lasseter, V.K., Wolyniec, P.S., Nestadt, G., Thornquist, M., Ullrich, G., McGrath, J., Kasch, L., Lamacz, M., Thomas, M.G., Gehrig, C., Radhakrishna, U., Snyder, S.E., Balk, K.G., Neufeld, K., Swartz, K.L., DeMarchi, N., Papadimitriou, G.N., Dikeos, D.G., Stefanis, C.N., Chakravarti, A., Childs, B., Housman, D.E., Kazazian, H.H., Antonarakis, S.E., Pulver, A.E., 1998. Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. Nature Genetics 20, 70–73.

Brzustowicz, L.M., Honer, W.G., Chow, E.W., Little, D., Hogan, J., Hodgkinson, K., Bassett, A.S., 1999. Linkage of familial schizophrenia to chromosome 13q32. American Journal of Human Genetics 65, 1096–1103.

Cloninger, C.R., Kaufmann, C.A., Faraone, S.V., Malaspina, D., Svrakic, D.M., Harkavy-Friedman, J., Suarez, B.K., Matise, T.C., Shore, D., Lee, H., Hampe, C.L., Wynne, D., Drain, C., Markel, P.D., Zambuto, C.T., Schmitt, K., Tsuang, M.T., 1998. Genome-wide search for schizophrenia susceptibility loci: the NIMH Genetics Initiative and Millennium Consortium. American Journal of Medical Genetics 81, 275–281.

Concannon, P., Gogolin-Ewens, K.J., Hinds, D.A., Wapelhorst, B., Morrison, V.A., Stirling, B., Mitra, M., Farmer, J., Williams, S.R., Cox, N.J., Bell, G.I., Risch, N., Spielman, R.S., 1998. A second-generation screen of the human genome for susceptibility to insulin-dependent diabetes mellitus. Nature Genetics 19, 292–296.

Coon, H., Holik, J., Hoff, M., Reimherr, F., Wender, P., Myles-Worsley, M., Waldo, M., Freedman, R., Byerley, W., 1994. Analysis of chromosome 22 markers in nine schizophrenia pedigrees. American Journal of Medical Genetics 54, 72–79.

Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., Pericak-Vance, M.A., 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261, 921–923.

Davies, J.L., Kawaguchi, Y., Bennett, S.T., Copeman, J.B., Cordell, H.J., Pritchard, L.E., Reed, P.W., Gough, S.C.L., Jenkins, S.C., Palmer, S.M., Balfour, K.M., Rowe, B.R., Farrall, M., Barnett, A.H., Bain, S.C., Todd, J.A., 1994. A genome-wide search for human type 1 diabetes susceptibility genes. Nature 371, 130–136.

Durner, M., Vieland, V.J., Greenberg, D.A., 1999. Further evidence for the increased power of LOD scores compared with nonparametric methods. American Journal of Human Genetics 64, 281–289.

Faraone, S.V., Blehar, M., Pepple, J., Moldin, S.O., Norton, J., Nurnberger, J.I., Malaspina, D., Kaufmann, C.A., Reich, T., Cloninger, C.R., DePaulo, J.R., Berg, K., Gershon, E.S., Kirch, D.G., Tsuang, M.T., 1996. Diagnostic accuracy and confusability analyses: an application to the Diagnostic Interview for Genetic Studies. Psychological Medicine 26, 401–410.

Faraone, S.V., Matise, T., Svrakic, D., Pepple, J., Malaspina, D., Suarez, B., Hampe, C., Zambuto, C.T., Schmitt, K., Meyer, J., Markel, P., Lee, H., Harkavy Friedman, J., Kaufmann, C., Cloninger, C.R., Tsuang, M.T., 1998. Genome scan of European-American schizophrenia pedigrees: results of the NIMH Genetics Initiative and Millennium Consortium. American Journal of Medical Genetics 81, 290–295.

Goate, A., Chartier-Harlin, M.C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L.,

Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M., Roses, A., Williamson, R., Rossor, M., Owen, M., Hardy, J., 1991. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349, 704–706.

Hall, J.M., Lee, M.K., Newman, B., Morrow, J.E., Anderson, L.A., Huey, B., King, M.C., 1990. Linkage of early-onset familial breast cancer to chromosome 17q21. Science 250, 1684–1689.

Hanis, C.L., Boerwinkle, E., Chakraborty, R., Ellsworth, D.L., Concannon, P., Stirling, B., Morrison, V.A., Wapelhorst, B., Spielman, R.S., Gogolin-Ewens, K.J., Shepard, J.M., Williams, S.R., Risch, N., Hinds, D., Iwasaki, N., Ogata, M., Omori, Y., Petzold, C., Rietzch, H., Schroder, H.E., Schulze, J., Cox, N.J., Menzel, S., Boriraj, V.V., Chen, X., Lim, L.R., Lindner, T., Mereu, L.E., Wang, Y.Q., Xiang, K., Yamagata, K., Yang, Y., Bell, G.I., 1996. A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. Nature Genetics 13, 161–166.

Hashimoto, L., Habita, C., Beressi, J.P., Delepine, M., Besse, C., Cambon-Thomsen, A., Deschamps, I., Rotter, J.I., Djoulah, S., James, M.R., Froguel, P., Weissenbach, J., Lathrop, G.M., Julier, C., 1994. Genetic mapping of a susceptibility locus for insulin-dependent diabetes mellitus on chromosome 11q. Nature 371, 161–164.

Horikawa, Y., Oda, N., Cox, N.J., Li, X., Orho-Melander, M., Hara, M., Hinokio, Y., Lindner, T.H., Mashima, H., Schwarz, P.E., del Bosque-Plata, L., Oda, Y., Yoshiuchi, I., Colilla, S., Polonsky, K.S., Wei, S., Concannon, P., Iwasaki, N., Schulze, J., Baier, L.J., Bogardus, C., Groop, L., Boerwinkle, E., Hanis, C.L., Bell, G.I., 2000. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nature Genetics 26, 163–175.

Kaufmann, C.A., Suarez, B., Malaspina, D., Pepple, J., Svrakic, D., Markel, P.D., Meyer, J., Zambuto, C.T., Schmitt, K., Matise, T.C., Harkavy Friedman, J.M., Hampe, C., Lee, H., Shore, D., Wynne, D., Faraone, S.V., Tsuang, M.T., Cloninger, C.R., 1998. NIMH Genetics Initiative Millenium Schizophrenia Consortium: linkage analysis of African-American pedigrees. American Journal of Medical Genetics 81, 282–289.

Kendler, K.S., MacLean, C.J., O'Neill, F.A., Burke, J., Murphy, B., Duke, F., Shinkwin, R., Easter, S.M., Webb, B.T., Zhang, J., Walsh, D., Straub, R.E., 1996. Evidence for a schizophrenia vulnerability locus on chromosome 8p in the Irish Study of High-Density Schizophrenia Families. American Journal of Psychiatry 153, 1534–1540.

Kruglyak, L., Daly, M.J., Reeve-Daly, M.P., Lander, E.S., 1996. Parametric and nonparametric linkage analysis: a unified multipoint approach. American Journal of Human Genetics 58, 1347–1363.

Lander, E., Kruglyak, L., 1995. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nature Genetics 11, 241–247.

Levy-Lahad, E., Wijsman, E.M., Nemens, E., Anderson, L., Goddard, K.A., Weber, J.L., Bird, T.D., Schellenberg, G.D., 1995. A familial Alzheimer's disease locus on chromosome 1. Science 269, 970–973.

Mahtani, M.M., Widen, E., Lehto, M., Thomas, J., McCarthy, M., Brayer, J., Bryant, B., Chan, G., Daly, M., Forsblom, C., Kanninen, T., Kirby, A., Kruglyak, L., Munnelly, K., Parkkonen, M., Reeve-Daly, M.P., Weaver, A., Brettin, T., Duyk, G., Lander, E.S., Groop, L.C., 1996. Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. Nature Genetics 14, 90–94.

Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P.A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L.M., Ding, W., Bell, R., Rosenthal, J., Hussey, C., Tran, T., McClure, M., Frye, C., Hattier, T., Phelps, R., Haugen-Strano, A., Katcher, H., Yakumo, K., Gholami, Z., Shaffer, D., Stone, S., Bayer, S., Wray, C., Bogden, R., Dayananth, P., Ward, J., Tonin, P., Narod, S., Bristow, P.K., Norris, F.H., Helvering, L., Marrison, P., Rosteck, P., Lai, M., Barrett, J.C., Lewis, C., Neuhausen, S., Cannon-Albright, L., Goldgar, D., Wiseman, R., Kamb, A., Skolnic, M.H., 1994. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266, 66–71.

Narod, S.A., Feunteun, J., Lynch, H.T., Watson, P., Conway, T., Lynch, J., Lenoir, G.M., 1991. Familial breast–ovarian cancer locus on chromosome 17q12–q23. Lancet 338, 82–83.

Nurnberger Jr., J.I., Blehar, M.C., Kaufmann, C.A., York-Cooler, C., Simpson, S.G., Harkavy-Friedman, J., Severe, J.B., Malaspina, D., Reich, T., 1994. Diagnostic Interview for Genetic Studies. Rationale, unique features, and training. NIMH Genetics Initiative. Archives of General Psychiatry 51, 849–859 (discussion 863–844).

Pericak-Vance, M.A., Bebout, J.L., Gaskell Jr., P.C., Yamaoka, L.H., Hung, W.Y., Alberts, M.J., Walker, A.P., Bartlett, R.J., Haynes, C.A., Welsh, K.A., Earl, N.L., Heyman, A., Clark, C.M., Roses, A.D., 1991. Linkage studies in familial Alzheimer disease: evidence for chromosome 19 linkage. American Journal of Human Genetics 48, 1034–1050.

Pulver, A.E., Lasseter, V.K., Kasch, L., Wolyniec, P., Nestadt, G., Blouin, J.L., Kimberland, M., Babb, R., Vourlis, S., Chen, H., Chen, H., Lalioti, M., Morris, M.A., Karayiorgou, M., Ott, J., Meyers, D., Antonarakis, S.E., Housman, D., Kazazian, H.H., 1995. Schizophrenia: a genome scan targets chromosomes 3p and 8p as potential sites of susceptibility genes. American Journal of Medical Genetics 60, 252–260.

Riley, B.P., McGuffin, P., 2000. Linkage and associated studies of schizophrenia. American Journal of Medical Genetics 97, 23–44.

Sawa, A., Snyder, S.H., 2002. Schizophrenia: diverse approaches to a complex disease. Science 296, 692–695.

Schellenberg, G.D., Bird, T.D., Wijsman, E.M., Orr, H.T., Anderson, L., Nemens, E., White, J.A., Bonnycastle, L., Weber, J.L., Alonso, M.E., Potter, H., Heston, L.L., Martin, G.M., 1992. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. Science 258, 668–671.

St. George-Hyslop, P.H., Tanzi, R.E., Polinsky, R.J., Haines, J.L., Nee, L., Watkins, P.C., Myers, R.H., Feldman, R.G., Pollen, D., Drachman, D., Growdon, J., Bruni, A., Foncin, J.F., Salmon, D., Frommelt, P., Amaducci, L., Sorbi, S., Piacentini, S., Stewart, G.D., Hobbs, W.J., Conneally, P.M., Gusella, J.F., 1987. The genetic defect causing familial Alzheimer's disease maps on chromosome 21. Science 235, 885–890.

Stoffers, D.A., Ferrer, J., Clarke, W.L., Habener, J.F., 1997. Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. Nature Genetics 17, 138–139.

Straub, R.E., MacLean, C.J., Ma, Y., Webb, B.T., Myakishev, M.V., Harris-Kerr, C., Wormley, B., Sadek, H., Kadambi, B., O'Neill, F.A., Walsh, D., Kendler, K.S., 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. Molecular Psychiatry 7, 542–559.

Tsuang, M.T., 2000. Schizophrenia: genes and environment. Biological Psychiatry 47, 210–220.

Tsuang, M.T., Faraone, S.V., 1995. The case for heterogeneity in the etiology of schizophrenia. Schizophrenia Research 17, 161–175.

Tsuang, M.T., Lyons, M.J., Faraone, S.V., 1990. Heterogeneity of schizophrenia. Conceptual models and analytic strategies. British Journal of Psychiatry 156, 17–26.

Vionnet, N., Stoffel, M., Takeda, J., Yasuda, K., Bell, G.I., Zouali, H., Lesage, S., Velho, G., Iris, F., Passa, P., Froguel, P., Cohen, D., 1992. Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. Nature 356, 721–722.

Yamagata, K., Furuta, H., Oda, N., Kaisaki, P.J., Menzel, S., Cox, N.J., Fajans, S.S., Signorini, S., Stoffel, M., Bell, G.I., 1996a. Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). Nature 384, 458–460.

Yamagata, K., Oda, N., Kaisaki, P.J., Menzel, S., Furuta, H., Vaxillaire, M., Southam, L., Cox, R.D., Lathrop, G.M., Boriraj, V.V., Chen, X., Cox, N.J., Oda, Y., Yano, H., Le Beau, M.M., Yamada, S., Nishigori, H., Takeda, J., Fajans, S.S., Hattersley, A.T., Iwasaki, N., Hansen, T., Pedersen, O., Polonsky, K.S., Turner, R.C., Velho, G., Chevre, J.C., Froguel, P., Bell, G.I., 1996b. Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). Nature 384, 455–458.