Molecular Psychiatry (2006), 1–8

www.nature.com/mp

ORIGINAL ARTICLE

Identification of the semaphorin receptor PLXNA2 as a candidate for susceptibility to schizophrenia

S Mah[1,4], MR Nelson[1,4], LE DeLisi[2], RH Reneland[1], N Markward[1], MR James[3], DR Nyholt[3], N Hayward[3], H Handoko[3], B Mowry[3], S Kammerer[1] and A Braun[1]

[1]*Sequenom Inc., San Diego, CA, USA;* [2]*Department of Psychiatry, New York University, New York, NY, USA and* [3]*Queensland Institute of Medical Research, Herston, QLD, Australia*

The discovery of genetic factors that contribute to schizophrenia susceptibility is a key challenge in understanding the etiology of this disease. Here, we report the identification of a novel schizophrenia candidate gene on chromosome 1q32, plexin A2 (*PLXNA2*), in a genome-wide association study using 320 patients with schizophrenia of European descent and 325 matched controls. Over 25 000 single-nucleotide polymorphisms (SNPs) located within approximately 14 000 genes were tested. Out of 62 markers found to be associated with disease status, the most consistent finding was observed for a candidate locus on chromosome 1q32. The marker SNP rs752016 showed suggestive association with schizophrenia (odds ratio (OR) = 1.49, *P* = 0.006). This result was confirmed in an independent case–control sample of European Americans (combined OR = 1.38, *P* = 0.035) and similar genetic effects were observed in smaller subsets of Latin Americans (OR = 1.26) and Asian Americans (OR = 1.37). Supporting evidence was also obtained from two family-based collections, one of which reached statistical significance (OR = 2.2, *P* = 0.02). High-density SNP mapping showed that the region of association spans approximately 60 kb of the *PLXNA2* gene. Eight out of 14 SNPs genotyped showed statistically significant differences between cases and controls. These results are in accordance with previous genetic findings that identified chromosome 1q32 as a candidate region for schizophrenia. PLXNA2 is a member of the transmembrane semaphorin receptor family that is involved in axonal guidance during development and may modulate neuronal plasticity and regeneration. The PLXNA2 ligand semaphorin 3A has been shown to be upregulated in the cerebellum of individuals with schizophrenia. These observations, together with the genetic results, make *PLXNA2* a likely candidate for the 1q32 schizophrenia susceptibility locus.
Molecular Psychiatry advance online publication, 10 January 2006; doi:10.1038/sj.mp.4001785

Keywords: single-nucleotide polymorphism; genome-wide association; chromosome 1q32; plexin; semaphorin

Introduction

Schizophrenia is one of the most severe and disabling of the mental disorders affecting up to 1% of the world's population, regardless of gender, race, culture and class.[1] Family, twin and adoption studies strongly suggest a genetic basis for this illness with the heritability estimated at about 70%.[2] Among the genes that have been postulated as candidates from linkage and association studies are the regulator of G-protein signaling 4 on 1q22,[3] disrupted in schizophrenia (DISC) 1 and 2 on chromosome 1q42,[4] dystrobrevin-binding protein 1 on 6p22,[5] neuregulin 1 on 8p21,[6] D-amino-acid oxidase activator (G72) on 13q34,[7] catechol-*O*-methyltransferase on 22q11[8] and proline dehydrogenase 1 on 22q11.[9] Despite intensive research, results for most of these regions have been inconsistent.[10,11]

There has been increasing interest in using whole-genome association as a method to identify genes involved in complex diseases. Genome-wide association studies using large numbers of single-nucleotide polymorphisms (SNPs) have been proposed and successfully applied to find genetic variations associated with myocardial infarction,[12] bone mineral density[13] and breast cancer.[14,15] In an effort to identify genes and variants that influence the risk of schizophrenia, we carried out a large-scale SNP association study using a multi-center collection of schizophrenia cases and controls. We identified a gene on chromosome 1q32, plexin A2 (*PLXNA2*), as a candidate gene for schizophrenia. Several linkage studies previously reported schizophrenia susceptibility loci in the 1q22-42 region;[16–20] other studies, however, have been

Correspondence: Dr A Braun, Sequenom Inc., 3595 John Hopkins Court, San Diego, CA 92121, USA.
E-mail: andibraun@earthlink.net
[4]These authors contributed equally to this work.
Received 17 May 2005; revised 19 October 2005; accepted 16 November 2005

unable to reproduce these results.[21–23] The findings described here might be important for the elucidation of this controversial schizophrenia locus.

Materials and methods

Schizophrenia collections
Subjects were selected from four clinical DNA sample collections that had been collected independently for genetic research. The appropriate research ethics committees approved all collections and all subjects gave their written informed consent to have blood drawn and to participate in genetic studies before enrollment. Patient information was collected, stored and analyzed in accordance with the highest ethical standards.

Discovery subjects
The affected subjects were derived from a large multicenter collection of patients with schizophrenia recruited by Precision Medicine Inc. (PMI). All individuals qualified for a clinical trial of the new atypical antipsychotic medication olanzapine. All subjects had DSM-IV diagnoses of paranoid schizophrenia (295.30), disorganized schizophrenia (295.10), residual schizophrenia (295.60), undifferentiated schizophrenia (295.90) or schizoaffective disorder (295.70). The majority of subjects were collected in Los Angeles, with additional subjects coming from Atlanta, Ohio, Philadelphia and Austin. Diagnoses were confirmed and documented by the psychiatrist in charge of the patient. Additional inclusion criteria were >6 months of olanzapine treatment (before enrollment) and absence of other antipsychotic drug treatments. The cases selected for our genetic study consisted of 110 females and 210 males of European descent, with a median age of 48 and 45, respectively. The numbers of affected male (M) and female (F) individuals were as follows: schizoaffective disorder 63 M/50 F, paranoid schizophrenia 97 M/39 F, undifferentiated schizophrenia 47 M/19 F, residual schizophrenia 1 M/1 F and disorganized schizophrenia 2 M/1 F. Three hundred twenty-five controls were selected from a repository of healthy blood donors from the California Blood Bank of San Bernardino and Riverside Counties. Subjects with a family history of neuropsychiatric disorders were excluded and controls were sex and age matched to cases.

Replication subjects
The affected subjects for the replication sample consisted of 200 European Americans, 157 African Americans, 111 Latin Americans and 63 Asian Americans derived from a large multi-center collection of patients with schizophrenia collected by PMI as described for the discovery subjects. The age and sex distribution in all ethnic groups was similar to the discovery sample. Unrelated controls of the appropriate ethnicities were selected from the blood donor collection described above utilizing the same matching criteria as in the discovery sample. The number of control individuals in the four ethnic groups was 230, 180, 123 and 72, respectively. No detailed information on the ancestral origin of the two sample sets collected by PMI was available.

Family-based sample 1
A family-based sample was selected from a collection of 294 affected sibling pair nuclear families from throughout the USA (a national sample), and centers in the UK (Oxford), Italy (Milan) and Chile (Santiago) recruited between 1985 and 2000.[21] The large majority of families (263) were from the USA and the UK and were predominantly of Northern European descent. The sample used in this study consisted of 463 affected and unaffected individuals from 135 families with at least two siblings diagnosed with schizophrenia or schizoaffective disorder. The two largest ethnic groups included European Americans (397 individuals from 117 families) and Latin Americans (45 individuals from 13 families). Diagnoses were made using DSM-III-R criteria on the basis of medical records and structured interviews performed by trained professionals using validated diagnostic instruments.

Family-based sample 2
A second family-based sample originated from an Australian high-density family study that consisted of 96 recruited families. Each family had to include a proband with DSM-III-R or DSM-IV schizophrenia plus at least one other person affected with either schizophrenia or schizoaffective disorder. Eighty-five of these families were part of a larger molecular genetic study of schizophrenia, the NIMH GI SZ MGS1, a collaboration of 10 centers (nine US and one Australian).[24] Participants were recruited from clinical networks and advocacy groups such as Schizophrenia Fellowship. Individuals (18 years or older) were interviewed by trained research clinicians using the Diagnostic Interview for Genetic Studies 2.0 (DIGS),[25] a semistructured interview specifically constructed for the assessment of psychotic and major mood disorders. Diagnoses were based on the DIGS interview, family history information from a reliable informant using the Family Interview for Genetic Studies.[26,27] Two experienced research psychiatrists independently reviewed these data and assigned a diagnosis, subsequently meeting to resolve any diagnostic disagreements (by reference to collected data or by collection of additional critical clinical data) and thus to assign a primary Best Estimate Final Diagnosis.[28] The collection was composed of 370 individuals, including 177 affected and 193 unaffected individuals from 90 affected sibling pairs and six multiplex families (at least three affected individuals).[29,30] Ninety-four families were of European, one of Australian Aboriginal/Melanesian and one of Asian descent.

SNP markers and genotyping
A set of 25 494 SNP markers located within approximately 14 000 genes was selected from a collection of

125 799 experimentally validated polymorphisms.[31] The selection criteria for the SNP set were location within a gene coding regions, a minor allele frequency greater than 0.02 (95% have frequencies greater than 0.1), and an inter-marker spacing of about 40 kb. SNP annotation is based on the National Center for Biotechnology Information (NCBI) dbSNP database, RefSNP, Build 118. Genomic annotation is based on NCBI Genome Build 34. Gene annotation is based on Entrez Gene entries for which NCBI was providing positions on the Mapview FTP site.

For pooled DNA assays, 25 ng of case and control DNA pools was used for amplification at each site. All PCR and MassEXTEND™ reactions were conducted using standard conditions.[32,33] Relative allele frequency estimates from each pooled collection were derived from area under the peak calculations of mass spectrometry measurements from four analyte aliquots as described elsewhere.[32–34] For individual genotyping, the same procedure was applied except only 2.5 ng DNA was used and only one mass spectrometry measurement was taken.

PLXNA2 resequencing

Exons 10–17 of the *PLXNA2* gene were re-sequenced using 18 randomly chosen DNAs from individuals with schizophrenia from the discovery sample. DNA sequence from PCR amplicons corresponding to the exons and 100 bp of flanking intronic sequences was determined by ABI 3730 sequencing performed at Retrogen Inc. (San Diego, CA, USA). Contig assembly and SNP identification were determined using Sequencher (Gene Codes Corporation, Ann Arbor, MI, USA).

Statistical analysis

Tests of association between cases and controls in each SNP using pooled DNA were carried out as explained elsewhere.[35] Sources of measurement variation included pool formation, PCR/mass extension and chip measurement. When three or more replicate measurements of an SNP were available within a model level, the corresponding variance component was estimated from the data. Otherwise, the following historical laboratory averages were used: pool formation = 5.0×10^{-5}, PCR/mass extension = 1.7×10^{-4} and chip measurement = 1.0×10^{-4}. Tests of association using individual genotypes used a χ^2 test of heterogeneity based on allele and genotype frequencies. No attempt was made to correct *P*-values for multiple testing. Rather, *P*-values are provided to compare the relative strength of association. *P*-values less than 0.05 are referred to as statistically significant. Odds ratios (ORs) were calculated using the major allele frequency. The transmission disequilibrium test (TDT) was performed using the latest version of TDTPHASE, part of the UNPHASED package.[36] For affected sib-pair families, all affected offspring in each family were analyzed using the TDTPHASE Haplotype-based Haplotype Relative Risk (HHRR) approach (*-EM* option); missing parental genotypes were not imputed. Therefore, to investigate the effect of non-independence of the transmissions – owing to the use of multiple patients from the same family – we permuted *P*-values (10 000 replicates) using the same permuted transmission status for siblings (*-permutation 10 000* and *-robustperm* options), which represents a valid association test in the presence of linkage.

Results

Initial large-scale association analysis

We performed a genome-wide association study using 25 494 SNPs located within 10 kb of 14 000 Entrez Gene annotated genes and a sample consisting of 320 schizophrenia cases and 325 matched controls. To facilitate the screening of such a large number of SNPs, we employed a high-throughput approach that used DNA pools, chip-based mass spectrometry[32–34] and a three-step SNP selection process that had been successfully used previously.[13–15] In the first step, we performed a single PCR and primer extension reaction for each SNP on two DNA pools consisting of schizophrenia cases and controls, respectively. Relative allele frequencies obtained from four mass spectrometry measurements of each extension product were compared between pools. In the second step, the 1457 SNPs (6%) with the statistically most significant associations ($P \leqslant 0.02$) were re-measured in triplicate on each DNA pool. In the third step, we genotyped the 95 most significant SNPs ($P \leqslant 0.02$) from step two on all individuals comprising the pools. Based on the genotype results, 62 SNPs were confirmed to have statistically significant allele frequency differences between cases and controls ($P < 0.05$).

Identification of the PLXNA2 locus

Genome-wide studies using tens of thousands of SNPs and liberal statistical selection criteria are expected to yield a high proportion of false-positive associations. In addition, the discovery and replication samples were collected in North America, which is ethnically diverse and differences in allele frequencies may therefore result from stratification artifacts. To distinguish true genetic effects from false-positive associations, the 62 selected SNPs were genotyped in an additional case–control sample. The most consistent effect was observed for rs752016, a C/T polymorphism within intron 11 of *PLXNA2* on chromosome 1q32. For the initial discovery sample, the minor allele frequency for the C allele was 0.22 in the control group and 0.16 in the case group (OR = 1.49; $P = 0.006$). In the replication sample, the results of the association test in the European American subset was very similar to those in the discovery group with a *P*-value of 0.07 and an OR of 1.38 (Table 1). The analysis of the discovery sample and the European American subset of the replication sample resulted in a combined significance of $P = 0.035$ (OR = 1.38). Similar genetic effects were observed in Latin Americans (OR = 1.26) and Asian

Table 1 Analysis of association of SNPs in *PLXNA2* with susceptibility to schizophrenia

SNP	*Sample*	N[a] *(Cas/Con)*	*HWE*[b]	*AF*[c]	*OR*[d]	P-*value*[e]	*Genotype frequencies (Cas/Con)*[f]			P-*value*[g]
							CC	*CT*	*TT*	
rs752016 marker (intronic)	Discovery – EurAm	304/323	0.64	0.16/0.22	1.49	0.006	0.03/0.05	0.26/0.33	0.71/0.62	0.026
	Replication – EurAm	182/226	0.16	0.17/0.22	1.38	0.07	0.03/0.03	0.27/0.38	0.69/0.59	0.078
	Replication – LatAm	100/111	0.43	0.18/0.22	1.26	0.35	0.04/0.06	0.28/0.31	0.68/0.63	0.650
	Replication – AfrAm	147/177	0.73	0.07/0.07	0.95	0.86	0.0/0.01	0.14/0.12	0.86/0.87	0.590
	Replication – AsnAm	57/71	0.83	0.39/0.47	1.37	0.22	0.14/0.21	0.51/0.52	0.35/0.27	0.450
	Family-based 1	114/114		0.12/0.15	1.31	0.34				
	Family-based 2	139/139		0.21/0.19	0.87	0.52				
							AA	AG	GG	
rs841865 (intronic)	Discovery – EurAm	306/323	0.40	0.16/0.22	1.47	0.008	0.03/0.04	0.27/0.37	0.70/0.59	0.018
	Replication – EurAm	188/226	0.92	0.15/0.21	1.51	0.03	0.02/0.04	0.27/0.34	0.71/0.62	0.068
	Replication – LatAm	100/111	0.91	0.12/0.12	1.02	0.95	0.03/0.01	0.17/0.22	0.80/0.77	0.400
	Replication – AfrAm	149/178	0.59	0.05/0.04	0.67	0.29	0.0/0.0	0.11/0.07	0.89/0.93	0.280
	Replication – AsnAm	56/71	0.81	0.30/0.31	1.03	0.91	0.12/0.08	0.36/0.45	0.52/0.46	0.510
	Family-based 1	102/102		0.06/0.11	2.24	0.02				
	Family-based 2	Not tested								
							CC	*CG*	*GG*	
rs1327175 (intronic)	Discovery – EurAm	316/319	0.74	0.05/0.08	1.74	0.02	0.91/0.85	0.09/0.15	0.0/0.01	0.045
	Replication – EurAm	188/221	0.67	0.03/0.06	2.16	0.03	0.95/0.88	0.05/0.12	0.01/0.0	0.018
	Replication – LatAm	99/110	0.80	0.06/0.11	1.89	0.08	0.88/0.80	0.12/0.18	0.0/0.02	0.180
	Replication – AfrAm	146/177	0.15	0.02/0.01	0.53	0.34	0.96/0.98	0.04/0.02	0.0/0.0	0.340
	Replication – AsnAm	56/68	0.71	0.09/0.10	1.17	0.72	0.82/0.79	0.18/0.21	0.0/0.0	0.700
	Family-based 1	109/109		0.04/0.04	1	1				
	Family-based 2	145/145		0.04/0.07	1.62	0.2				
							TT	*TC*	*CC*	
rs2498028 (intronic)	Discovery – EurAm	312/323	0.91	0.13/0.20	1.65	0.001	0.74/0.64	0.26/0.32	0.0/0.04	0.001
	Replication – EurAm	181/228	0.65	0.14/0.19	1.46	0.05	0.73/0.64	0.25/0.32	0.02/0.03	0.130
	Replication – LatAm	102/110	0.70	0.11/0.10	0.92	0.79	0.81/0.81	0.16/0.18	0.03/0.01	0.510
	Replication – AfrAm	145/174	0.18	0.04/0.02	0.6	0.27	0.92/0.95	0.08/0.05	0.0/0.0	0.260
	Replication – AsnAm	54/68	0.56	0.27/0.33	1.35	0.29	0.57/0.43	0.31/0.49	0.11/0.09	0.160
	Family-based 1	105/105		0.06/0.13	1.86	0.08				
	Family-based 2	Not tested								

Eur = European; Lat = Latin; Afr = African; Asn = Asian; Am = American.

[a]Number of unrelated cases and controls for association tests. For family-based tests, the number of equivalent cases and controls (i.e. rs1327175 for family-based 2: 290 allelic transmissions/non-transmissions to affected children = 145 cases and 145 controls).

[b]*P*-value of Hardy–Weinberg proportions in controls with Yates continuity correction.

[c]AF refers to minor relative allele frequency in the unrelated cases and controls, respectively, and the frequency of the transmitted and non-transmitted allele, respectively, in the TDT analysis of the family-based samples.

[d]Odds ratio with reference to allele that increases in frequency in unrelated cases or the over transmission odds ratio derived from the TDT in family-based analyses using the same reference allele.

[e]*P*-value of test comparing allele frequencies between unrelated cases and controls or *P*-value of the TDT for the family-based tests.

[f]Relative genotype frequencies.

[g]*P*-value of test comparing genotype frequencies between cases and controls.

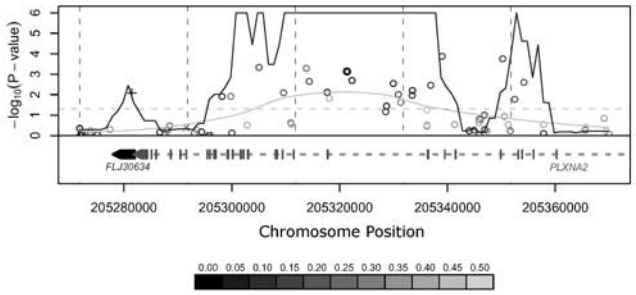

Figure 1 Pool-based association fine mapping of schizophrenia susceptibility locus on chromosome 1q32. Sixty-eight SNPs in a 100-kb window were tested and compared between pooled discovery cases and controls. The *x*-axis corresponds to the chromosomal position and the *y*-axis to the test *P*-value (shown on the $-\log_{10}$ scale). The continuous dark line represents a goodness-of-fit test for excess of significance (compared to the 0.05 proportion expected by chance alone) in a 10-kb sliding window assessed at 1-kb increments. The continuous light gray line is the result of a non-linear smoothing function showing a weighted average of the *P*-values across the region. The shade of each point corresponds to the minor allele frequency of the corresponding SNP in the control sample (see legend below graph). The shape of each point depicts the SNP as non-synonymous ('+'), synonymous ('x') or intronic/intergenic ('o'). The Entrez Gene annotation for NCBI genome Build 34 is included.

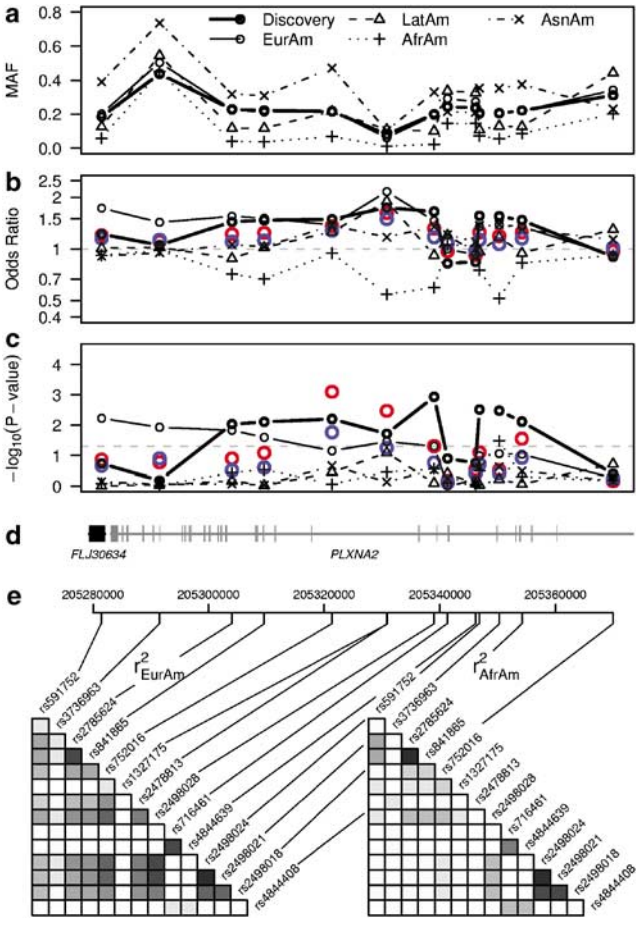

Figure 2 Genotype analysis of 14 SNPs in an 88-kb region in the 3′ region of *PLXNA2*. Minor allele frequencies, ORs and *P*-values (−log10) are plotted by SNP chromosome position, stratified by ethnicity (see **a**, top insert). (**a**) Minor allele frequencies (MAF) are given for the control sample of each subgroup. The reference allele was determined by the minor allele in the discovery sample controls. (**b**) ORs compare the minor allele in cases versus controls. (**c**) *P*-values are derived from the χ^2-test of independence from the two-by-two allele-based contingency table. Red circles represent the total combined *P*-value in all samples; the blue circles represent the combined *P*-value in the replication samples alone. (**d**) Gene map and chromosomal positions as in Figure 1. SNP locations are indicated as tick marks. (**e**) Representative estimates of pairwise linkage disequilibrium (LD expressed as r2) in the European and African American replication samples. LD values are represented as gray-scale ranging from white (LD = 0) to black (LD = 1) at increments of 0.1.

Americans (OR = 1.37); however, owing to small sample numbers in these groups, statistical significance was not achieved. No genetic effect was observed in African Americans, in which the SNP had the lowest allele frequency (0.07) (Table 1). Noteworthy, none of the 62 markers found to be associated with schizophrenia in this study were associated with any previously reported candidates in the literature. However, the SNP set used only encompasses approximately 50% of all annotated genes with an inter-marker spacing of about 40 kb; negative results might therefore be attributed to the limitations of such a set.

Association fine mapping

To fine map the region of association, we analyzed an additional 67 SNPs in a 100-kb window surrounding the marker SNP (rs752016) in the discovery case and control pools (Figure 1). A goodness-of-fit test for excess of significance indicated that the region of association extended approximately 60 kb, spanning exons 5–26 of the *PLXNA2* gene; the excess of significance decreased towards the 5′- and 3′-ends of the gene.

Based on the fine-mapping results, 14 SNPs with varying levels of linkage disequilibrium (Figure 2e) were chosen for genotyping in the discovery and replication samples. All SNPs displayed similar allele frequencies in the European American subsets of both samples (Figure 2a). Association analysis showed that the region of highest significance was within 20 kb to either side of rs752016 (Figures 2b and c). Out of the 14 genotyped SNPs, three were statistically significant at the 0.05 level, four at the 0.01 and one at the 0.001 level. The results for four of the SNPs are shown in Table 1. Analysis of haplotypes consisting of all permutations of the 14 genotyped SNPs did not reveal any haplotype with a stronger association than individual SNPs (data not shown).

In order to identify potential causative SNPs, exons in the 40-kb region of highest association (exons 10–17) were re-sequenced in 18 affected individuals.

No non-synonymous SNPs were identified suggesting that the functional effect might be mediated by variations influencing RNA splicing or expression levels. Alternatively, non-synonymous variations could exist in exons that were not sequenced.

Family-based association analyses

In addition to the case–control analyses, we performed a family-based test in a multi-ethnic affected sib-pair collection (family-based 1). Based on the association fine-mapping and genotyping results obtained in the case–control studies, the four SNPs that best represented the region (rs752016, rs841865, rs1327175 and rs2498028) were genotyped in this collection. We used the TDT to evaluate the association between parents and affected individuals and the results are presented in Table 1. The number of allelic transmissions to affected individuals ranged from 204 to 227 with a mean of 220 for the four SNPs. A statistically significant association was observed for rs841865 ($P = 0.02$, OR = 2.24); rs2498028 showed borderline significance ($P = 0.08$, OR = 1.86). The marker SNP rs752016 did not reach statistical significance, but showed the expected trend.

A second affected sib-pair sample collected in Australia (family-based 2) was analyzed using six SNPs that were independently selected based on low linkage disequilibrium. These included two SNPs (rs752016 and rs1327175) that had also been genotyped in the other collections (Table 1). The number of allelic transmissions to affected individuals ranged from 273 to 289 with a mean of 280 for the six SNPs. None of the six SNPs were significantly associated with schizophrenia in this sample; however, the genetic effect observed for rs1327175 (OR = 1.62) showed the same direction as in the analysis of the European American case–control samples.

Discussion

In the current study, we have identified an association between variants in the *PLXNA2* gene and schizophrenia in two collections of schizophrenia cases and controls. This association was supported by results from the analysis of two family-based samples, one of which reached statistical significance. Additionally, we have used SNP association fine mapping to identify an approximately 60-kb region within the *PLXNA2* gene that is most strongly associated with schizophrenia. These data provide evidence that variants in the *PLXNA2* gene influence susceptibility for this complex genetic disorder.

PLXNA2 is a member of the semaphorin receptor family. Members of this family have been implicated in the development of axonal projections and in neural regeneration.[37–39] Plexins heterodimerize with members of the neuropilin (NRP) family to form receptors for the semaphorin family of secreted axonal chemorepellants.[40,41] Specifically, PLXNA2 heterodimerizes with either NRP1 or 2 and forms a functional receptor for the class 3 semaphorins.[40] The prevailing view is that schizophrenia is a disorder of neurodevelopment and plasticity, resulting in abnormalities of synaptic connectivity that may involve several neurotransmitters, including dopamine, glutamate and serotonin.[42] The pathophysiological symptoms of schizophrenia suggest a potential dysfunction in several brain regions that may also include neuronal and synaptic connections to temporal and frontal cortical regions, which are involved in higher cognitive functions, such as language and working memory.[43,44] In addition, MRI and post-mortem histopathological studies have revealed structural abnormalities in these cortical regions.[45,46] Interestingly, expression studies in mice showed a markedly reduced expression of PLXNA2 in the neocortex after birth, suggesting an important role in the development of neuronal connection patterns, especially in regions of the cortex.[47] Interestingly, the PLXNA2/NRP1 ligand, semaphorin 3A, was shown to be increased in the cerebellum of individuals with schizophrenia.[48]

Schizophrenia susceptibility genes have been proposed on many chromosomes including 1q, 5q, 6p, 6q, 8p, 10p, 13q, 18p and 22q (reviewed in O'Donovan *et al*.[11]). *PLXNA2* is located in the 1q32 region, a schizophrenia locus that was originally identified in a family-based linkage study[16] and has received support from several independent studies.[18–20] However, there have been several studies that did not confirm this locus.[21–23] This might be due to the inherent limitations of linkage studies, but could also be explained by the size and heterogeneity of samples used in the different studies.[49] This might also explain why our findings on *PLXNA2* could not be replicated in the family-based sample from Australia considering that the number of families represented in this set was relatively limited ($N = 96$).

In a linkage study using 221 Finnish schizophrenia families, Ekelund *et al*.[18] reported a strong evidence for linkage at D1S2709, which is an intragenic marker of the *DISC1* gene. Additional support for this gene stems from the identification of a translocation that segregates with schizophrenia and disrupts the *DISC1* and *2* genes.[50] These two genes have been the focus of some groups working on the 1q region, but it is yet unclear what fraction of the observed schizophrenia susceptibility they might account for.

PLXNA2 is located approximately 35 kb upstream of D1S2891, a marker for which Hovatta *et al*.[16] have observed a maximum pairwise LOD score of 3.82 in a linkage study for schizophrenia. In a subsequent fine-mapping project, the same group identified a marker (D1S245) with an LOD score of 2.3 that lies within 1 Mb of the *PLXNA2* gene.[18] Therefore, we conclude that variants in the *PLXNA2* gene might account for some of the observations in this region and might help resolve some of the inconsistencies. Given that *PLXNA2* and *DISC1/2* lie approximately 2.3 Mb apart, it is possible that the 1q32–42 region contains at least two schizophrenia loci, one of which might be explained by *PLXNA2* (1q32) and one by *DISC1/2* (1q42).

In addition to schizophrenia, the 1q32 locus has also been associated with bipolar disorder.[51] An LOD score of 2.6 was identified at marker GATA124F08, which is located about 550 kb upstream of *PLXNA2*. Family and twin studies have suggested hereditary overlap between bipolar disorder and schizophrenia; additionally, bipolar symptoms frequently overlap with those of other psychiatric disorders including schizophrenia.[52,53] The genetic findings presented here as well as the biological functions of PLXNA2 make it a likely candidate for the controversial 1q32 region. Further studies are needed to clarify if PLXNA2 is involved in the etiology of other neuropsychiatric diseases and whether specific mutations in the *PLXNA2* gene might be associated with these diseases.

Acknowledgments

We would like to thank the patients and family members who participated in this study, as well as the members of the Sequenom and QIMR genotyping teams for their support in producing the genetic data for this research. The majority of 'family-based sample 2' was recruited as part of the NIMH GI SZ MGS1 study. Data and biomaterials from these families were collected in 10 projects. The Principal Investigators and Co-Investigators were: Evanston Northwestern Healthcare/Northwestern University, Evanston, IL, USA, R01 MH59571, Pablo V Gejman, MD (Collaboration Coordinator; PI), Alan R Sanders, MD; Emory University School of Medicine, Atlanta, GA, USA, R01 MH59587, Farooq Amin, MD (PI); University of California, San Francisco, CA, USA, R01 MH60870, William F Byerley, MD (PI); University of Iowa, Iowa, IA, USA, R01 MH59566, Donald W Black, MD (PI), Raymond R Crowe, MD; Washington University, St Louis, MO, USA, R01 MH60879, C Robert Cloninger, MD (PI); University of Colorado, Denver, CO, USA, R01 MH59565, Robert Freedman, MD (PI), Ann Olincy, MD; University of Pennsylvania, Philadelphia, PA, USA, R01 MH61675, Douglas F Levinson MD (PI), and subcontract to Louisiana State University, New Orleans, LA, USA, Nancy G Buccola APRN, BC, MSN (subcontract PI); University of Queensland, Brisbane, Queensland, Australia, R01 MH59588, Bryan J Mowry, MD (PI); Mt Sinai School of Medicine, New York, NY, USA, R01 MH59586, Jeremy M Silverman, PhD (PI).

References

1 Jablensky A, Sartorius N. Is schizophrenia universal? *Acta Psychiatr Scand Suppl* 1988; **344**: 65–70.
2 Kendler KS. The genetics of schizophrenia: an overview. In: Tsuang MTaS JC (ed) *Handbook of Schizophrenia*. Amsterdam: Elsevier, 1988, pp 437–462.
3 Chowdari KV, Mirnics K, Semwal P, Wood J, Lawrence E, Bhatia T *et al.* Association and linkage analyses of *RGS4* polymorphisms in schizophrenia. *Hum Mol Genet* 2002; **11**: 1373–1380.
4 Blackwood DH, Fordyce A, Walker MT, St Clair DM, Porteous DJ, Muir WJ. Schizophrenia and affective disorders – cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. *Am J Hum Genet* 2001; **69**: 428–433.
5 Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV *et al.* Genetic variation in the 6p22.3 gene *DTNBP1*, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. *Am J Hum Genet* 2002; **71**: 337–348.
6 Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S *et al.* Neuregulin 1 and susceptibility to schizophrenia. *Am J Hum Genet* 2002; **71**: 877–892.
7 Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H *et al.* Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. *Proc Natl Acad Sci USA* 2002; **99**: 13675–13680.
8 Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE *et al.* Effect of *COMT* Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci USA* 2001; **98**: 6917–6922.
9 Liu H, Heath SC, Sobin C, Roos JL, Galke BL, Blundell ML *et al.* Genetic variation at the 22q11 *PRODH2/DGCR6* locus presents an unusual pattern and increases susceptibility to schizophrenia. *Proc Natl Acad Sci USA* 2002; **99**: 3717–3722.
10 Bray NJ, Owen MJ. Searching for schizophrenia genes. *Trends Mol Med* 2001; **7**: 169–174.
11 O'Donovan MC, Williams NM, Owen MJ. Recent advances in the genetics of schizophrenia. *Hum Mol Genet* 2003; **12**: R125–R133.
12 Ozaki K, Ohnishi Y, Iida A, Sekine A, Yamada R, Tsunoda T *et al.* Functional SNPs in the lymphotoxin-alpha gene that are associated with susceptibility to myocardial infarction. *Nat Genet* 2002; **32**: 650–654.
13 Reneland RH, Mah S, Kammerer S, Hoyal CR, Marnellos G, Wilson SG *et al.* Association between a variation in the phosphodiesterase 4D gene and bone mineral density. *BMC Med Genet* 2005; **6**: 9.
14 Kammerer S, Roth RB, Reneland R, Marnellos G, Hoyal CR, Markward NJ *et al.* Large-scale association study identifies *ICAM* gene region as breast and prostate cancer susceptibility locus. *Cancer Res* 2004; **64**: 8906–8910.
15 Kammerer S, Roth RB, Hoyal CR, Reneland R, Marnellos G, Kiechle M *et al.* Association of the *NuMA* region on chromosome 11q13 with breast cancer susceptibility. *Proc Natl Acad Sci USA* 2005; **102**: 2004–2009.
16 Hovatta I, Varilo T, Suvisaari J, Terwilliger JD, Ollikainen V, Arajarvi R *et al.* A genomewide screen for schizophrenia genes in an isolated Finnish subpopulation, suggesting multiple susceptibility loci. *Am J Hum Genet* 1999; **65**: 1114–1124.
17 Brzustowicz LM, Hodgkinson KA, Chow EW, Honer WG, Bassett AS. Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21–q22. *Science* 2000; **288**: 678–682.
18 Ekelund J, Hovatta I, Parker A, Paunio T, Varilo T, Martin R *et al.* Chromosome 1 loci in Finnish schizophrenia families. *Hum Mol Genet* 2001; **10**: 1611–1617.
19 Gurling HM, Kalsi G, Brynjolfson J, Sigmundsson T, Sherrington R, Mankoo BS *et al.* 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* 2001; **68**: 661–673.
20 Hwu HG, Liu CM, Fann CS, Ou-Yang WC, Lee SF. Linkage of schizophrenia with chromosome 1q loci in Taiwanese families. *Mol Psychiatry* 2003; **8**: 445–452.
21 DeLisi LE, Shaw SH, Crow TJ, Shields G, Smith AB, Larach VW *et al.* A genome-wide scan for linkage to chromosomal regions in 382 sibling pairs with schizophrenia or schizoaffective disorder. *Am J Psychiatry* 2002; **159**: 803–812.
22 Levinson DF, Holmans PA, Laurent C, Riley B, Pulver AE, Gejman PV *et al.* No major schizophrenia locus detected on chromosome 1q in a large multicenter sample. *Science* 2002; **296**: 739–741.
23 Maziade M, Fournier A, Phaneuf D, Cliche D, Fournier JP, Roy MA *et al.* Chromosome 1q12–q22 linkage results in eastern Quebec families affected by schizophrenia. *Am J Med Genet* 2002; **114**: 51–55.
24 Suarez BK, Duan J, Sanders A, Hinrichs CH, Jin CH, Buccola NG *et al.* Genomewide linkage scan of 409 European ancestry and African American families with schizophrenia: suggestive

evidence for linkage in 8p23.3–p12 and 11p11.2–q22.3 in the combined sample. *Am J Hum Genet*, (in press).
25 Nurnberger Jr JI, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J *et al.* Diagnostic interview for genetic studies. Rationale, unique features, and training. NIMH Genetics Initiative. *Arch Gen Psychiatry* 1994; **51**: 849–859 (discussion 863–864).
26 Gershon ES, DeLisi LE, Hamovit J, Nurnberger Jr JI, Maxwell ME, Schreiber J *et al.* A controlled family study of chronic psychoses. Schizophrenia and schizoaffective disorder. *Arch Gen Psychiatry* 1988; **45**: 328–336.
27 Maxwell ME. *Family Interview for Genetic Studies (FIGS): A Manual for FIGS*. Clinical Neurogenetics Branch, Intramural Research Program, National Institute of Mental Health: Bethesda, MD, 1992.
28 Leckman JF, Sholomskas D, Thompson WD, Belanger A, Weissman MM. Best estimate of lifetime psychiatric diagnosis: a methodological study. *Arch Gen Psychiatry* 1982; **39**: 879–883.
29 Mowry BJ, Ewen KR, Nancarrow DJ, Lennon DP, Nertney DA, Jones HL *et al.* Second stage of a genome scan of schizophrenia: study of five positive regions in an expanded sample. *Am J Med Genet* 2000; **96**: 864–869.
30 Mowry BJ, Holmans PA, Pulver AE, Gejman PV, Riley B, Williams NM *et al.* Multicenter linkage study of schizophrenia loci on chromosome 22q. *Mol Psychiatry* 2004; **9**: 784–795.
31 Nelson MR, Marnellos G, Kammerer S, Hoyal CR, Shi MM, Cantor CR *et al.* Large-scale validation of single nucleotide polymorphisms in gene regions. *Genome Res* 2004; **14**: 1664–1668.
32 Buetow KH, Edmonson M, MacDonald R, Clifford R, Yip P, Kelley J *et al.* High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Proc Natl Acad Sci USA* 2001; **98**: 581–584.
33 Bansal A, van den Boom D, Kammerer S, Honisch C, Adam G, Cantor CR *et al.* Association testing by DNA pooling: an effective initial screen. *Proc Natl Acad Sci USA* 2002; **99**: 16871–16874.
34 Mohlke KL, Erdos MR, Scott LJ, Fingerlin TE, Jackson AU, Silander K *et al.* High-throughput screening for evidence of association by using mass spectrometry genotyping on DNA pools. *Proc Natl Acad Sci USA* 2002; **99**: 16928–16933.
35 Barratt BJ, Payne F, Rance HE, Nutland S, Todd JA, Clayton DG. Identification of the sources of error in allele frequency estimations from pooled DNA indicates an optimal experimental design. *Ann Hum Genet* 2002; **66**(Parts 5–6): 393–405.
36 Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* 2003; **25**: 115–121.
37 Winberg ML, Noordermeer JN, Tamagnone L, Comoglio PM, Spriggs MK, Tessier-Lavigne M *et al.* Plexin A is a neuronal semaphorin receptor that controls axon guidance. *Cell* 1998; **95**: 903–916.
38 Cheng HJ, Bagri A, Yaron A, Stein E, Pleasure SJ, Tessier-Lavigne M. Plexin-A3 mediates semaphorin signaling and regulates the development of hippocampal axonal projections. *Neuron* 2001; **32**: 249–263.
39 Kikuchi K, Kishino A, Konishi O, Kumagai K, Hosotani N, Saji I *et al. In vitro* and *in vivo* characterization of a novel semaphorin 3A inhibitor, SM-216289 or xanthofulvin. *J Biol Chem* 2003; **278**: 42985–42991.
40 Tamagnone L, Artigiani S, Chen H, He Z, Ming GI, Song H *et al.* Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. *Cell* 1999; **99**: 71–80.
41 Rohm B, Ottemeyer A, Lohrum M, Puschel AW. Plexin/neuropilin complexes mediate repulsion by the axonal guidance signal semaphorin 3A. *Mech Dev* 2000; **93**: 95–104.
42 Sawa A, Snyder SH. Schizophrenia: diverse approaches to a complex disease. *Science* 2002; **296**: 692–695.
43 Harrison PJ. The neuropathology of schizophrenia. A critical review of the data and their interpretation. *Brain* 1999; **122**(Part 4): 593–624.
44 Innocenti GM, Ansermet F, Parnas J. Schizophrenia, neurodevelopment and corpus callosum. *Mol Psychiatry* 2003; **8**: 261–274.
45 Raine A, Harrison GN, Reynolds GP, Sheard C, Cooper JE, Medley I. Structural and functional characteristics of the corpus callosum in schizophrenics, psychiatric controls, and normal controls. A magnetic resonance imaging and neuropsychological evaluation. *Arch Gen Psychiatry* 1990; **47**: 1060–1064.
46 Frumin M, Golland P, Kikinis R, Hirayasu Y, Salisbury DF, Hennen J *et al.* Shape differences in the corpus callosum in first-episode schizophrenia and first-episode psychotic affective disorder. *Am J Psychiatry* 2002; **159**: 866–868.
47 Murakami Y, Suto F, Shimizu M, Shinoda T, Kameyama T, Fujisawa H. Differential expression of plexin-A subfamily members in the mouse nervous system. *Dev Dyn* 2001; **220**: 246–258.
48 Eastwood SL, Law AJ, Everall IP, Harrison PJ. The axonal chemorepellant semaphorin 3A is increased in the cerebellum in schizophrenia and may contribute to its synaptic pathology. *Mol Psychiatry* 2003; **8**: 148–155.
49 Macgregor S, Visscher PM, Knott S, Porteous D, Muir W, Millar K *et al.* Is schizophrenia linked to chromosome 1q? *Science* 2002; **298**: 2277, (author reply 2277).
50 Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA *et al.* Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum Mol Genet* 2000; **9**: 1415–1423.
51 Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G *et al.* A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. *Proc Natl Acad Sci USA* 1999; **96**: 5604–5609.
52 Moller HJ. Bipolar disorder and schizophrenia: distinct illnesses or a continuum? *J Clin Psychiatry* 2003; **64**(Suppl 6): 23–27 (discussion 28).
53 Berrettini W. Evidence for shared susceptibility in bipolar disorder and schizophrenia. *Am J Med Genet* 2003; **123C**: 59–64.