ORIGINAL ARTICLE

Anxiety and Comorbid Measures Associated With *PLXNA2*

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Context: Reduction in adult neurogenesis has been proposed as a mechanism for onset of depression. Semaphorins and their coreceptors, plexins, have been implicated in nervous system development and in adult neurogenesis. A recent genomewide association study of schizophrenia identified a variant of the gene encoding plexin A2 (*PLXNA2*) to be most consistently associated across study samples. Common genetic liabilities have been reported between psychiatric and psychological measures, but few examples exist of common genetic variants.

Objective: To perform a genetic association study between 6 single nucleotide polymorphisms from the *PLXNA2* gene (rs3736963, rs2767565, rs752016, rs1327175, rs2478813, and rs716461) and anxiety, depression, neuroticism, and psychological distress.

Design: Extreme discordant and concordant siblings.

Setting: Australia.

Participants: Study participants were selected with respect to extreme neuroticism scores from a population cohort of 18 742 twin individuals and their siblings. The participants and their parents (if blood or buccal samples were available) were genotyped, for a total of 2854 genotyped individuals from 990 families. Of these, 624 individuals with a diagnosis of anxiety or depression from 443 families were used in the association analysis.

Main Outcome Measures: All the participants completed the Composite International Diagnostic Interview, the 23-item Neuroticism scale of the revised Eysenck Personality Questionnaire, and the 10-item Kessler Psychological Distress Scale. Diagnoses of *DSM-IV* depression and anxiety were determined from the Composite International Diagnostic Interview.

Results: There was evidence of an allelic association between rs2478813 (and other single nucleotide polymorphisms correlated with it) and anxiety, depression, neuroticism, and psychological distress; the association with anxiety is significant after Bonferroni correction for multiple testing (empirical $P<.001$). The mouse ortholog of *PLXNA2* is located in a highly significant linkage region previously reported for anxiety in mice.

Conclusion: *PLXNA2* is a candidate for causal variation in anxiety and in other psychiatric disorders through its comorbidity with anxiety.

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FOR THE PAST 4 DECADES,[1,2] most theories of the etiology of major depression have centered on brain alterations in neurochemistry. Recently, a novel theory proposed that the waning and waxing of neurogenesis in the adult hippocampus is causal in the onset of and recovery from episodes of clinical depression.[3,4] Pivotal to this theory was the discovery that neurogenesis is not restricted to the developing brain but also occurs in the mature adult brain.[5] More recently, it has been shown that lithium (a commonly prescribed mood-stabilizing drug with antidepressant properties) enhances hippocampal neurogenesis[6] and, specifically, that neurogenesis is a requirement for the behavioral responses of antidepressants to be affected.[7] Therefore, genes that mediate hippocampal neurogenesis are plausible candidates for having risk variants for depression.

Semaphorins are members of a large, highly conserved family of molecular cues that have been implicated in the development of the nervous system, the guidance of axonal projections, axonal fasciculation, dendritic guidance, and neuronal migration.[8,9] In addition to their widespread expression during neuronal development, some semaphorins are persistently expressed in the adult nervous system,[8] are involved in neuronal apoptosis,[10] and have

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been shown to induce pruning of hippocampal axon branches in the mature brain.[11] Plexins are members of the semaphorin receptor family that act with neuropilins in mediating the effects of semaphorins.[12] Neuropilins have been found at synapses, suggesting that semaphorins also have a role as synaptic modulators.[13] Plexin A2 (*PLXNA2*), together with neuropilin 1 or 2, forms a functional receptor for class 3 semaphorins,[14] one of which, semaphorin 3A, has been found at elevated levels in patients with schizophrenia.[15] The functional role of semaphorins and their receptors makes them plausible candidates for variants to have a causal role in a broad range of psychiatric and psychological disorders.

Recently, a genomewide association study of schizophrenia that analyzed more than 25 000 single nucleotide polymorphisms (SNPs) from approximately 14 000 genes reported that, of 62 SNPs found to be associated in the "discovery" case-control set, 1 (accession ID rs752016 at dbSNP [see www.ncbi.nlm.nih.gov/projects/SNP]) showed consistency of association across replication samples (odds ratio [OR], 1.49; $P = .006$ in individuals of European descent). Fine mapping identified a region spanning exons 5 to 26 (approximately 60 kilobase [kb]) of the *PLXNA2* gene. As yet, it is unknown whether the *PLXNA2* variant associated with schizophrenia[16] might act during neuronal development, adult neurogenesis, or both. Currently, schizophrenia is viewed as a disorder of neurodevelopment and of synaptic activity regulated by neurotransmitters such as dopamine and serotonin.[17] However, the first study to implicate adult neurogenesis in schizophrenia has just been published,[18] inviting the hypothesis that common variants may affect adult neurogenesis in a spectrum of psychiatric disorders (although the same study found no evidence of neural stem cell proliferation, which is considered to be the first stage of adult neurogenesis in patients with major depression).

Under the Kraepelin[19] categorical disease model (and in *DSM-IV* and *International Statistical Classification of Diseases, 10th Revision*), schizophrenia and major depression are considered separate conditions. However, mounting evidence suggests that this clinically based distinction may not provide adequate guidance in attempting to elucidate the etiologic basis of these disorders.[20] There are few specific genetic risk factors that have been associated conclusively with both schizophrenia and depression, the most widely studied being the neuropathogenic role of brain-derived neurotrophic factor (reviewed by Angelucci et al[21]). Neuroticism, a dimension of personality that includes a tendency toward anxiety, depression, and low self-esteem, was found, when rated at age 16 years, to be associated with increased risk of later diagnosis of schizophrenia (OR, 1.93[22]). Neuroticism can be measured using self-report questionnaires such as the Eysenck Personality Questionnaire (EPQ),[23,24] and high EPQ neuroticism scores are associated with greater risk of early-onset depressive and anxiety disorders.[25] Neuroticism, depression, and anxiety are considered to have a common genetic basis.[26] The selection of participants with high EPQ neuroticism scores provided considerably enhanced odds of a range of related *DSM-IV* diagnoses, such as depression, social phobia, and generalized anxiety disorder[27] (in the study sample used in this article). In their review, Fanous and Kendler[28] concluded that, although neuroticism, depression, and schizophrenia may have shared specific genetic risk factors, more conclusive empirical evidence is needed.

Given (1) the comprehensive strategy used by Mah et al,[16] which resulted in their proposal of *PLXNA2* as a candidate gene for schizophrenia, (2) the possible shared etiology of schizophrenia, depression, and anxiety disorders, and (3) the function of plexins, which fits with the hypothesis that reduction in neurogenesis in the adult brain precipitates the onset of depression, we looked for associations between a set of 6 SNPs in *PLXNA2* and measures of neuroticism, psychological distress, depression, and anxiety. In this study, we show evidence of associations between *PLXNA2* SNPs and a spectrum of psychiatric and psychological traits, particularly driven by an association with anxiety, which shows a high degree of comorbidity with the other phenotypic measures. This study sample is unique because the same cohort of people, selected as sibling pairs concordant or discordant for extreme neuroticism scores, completed detailed questionnaires generating multiple phenotypic measures for each person, allowing us to follow up the initial association results to identify the phenotypic subtypes that drive the association in this sample.

METHODS

ASCERTAINMENT

This study was approved by the Queensland Institute of Medical Research human research ethics committee. Australian twin families recruited from the Australian Twin Registry completed self-report questionnaires between 1980 and 1995, including either the full 90-item EPQ revised (EPQ-R)[24] with a 23-item neuroticism scale or a shortened questionnaire (EPQ-Rs) with a 12-item neuroticism scale. The EPQ-R or EPQ-Rs neuroticism scores were available for 18 742 Australian twin individuals and their siblings. Sibling pairs that were either concordant or discordant for extreme EPQ scores (1 sibling in the top or bottom decile, the other sibling in the top or bottom quintile, and excluding monozygotic twin pairs) were recruited to complete a more detailed personality questionnaire. Using these criteria, multiple siblings were selected from some families. Blood (or buccal) samples were obtained where possible from the selected siblings and their parents. Full details of the recruitment procedure for this extreme discordant and concordant study, including response rates and incidence of *DSM-IV* diagnoses of anxiety- and depression-related disorders and longitudinal stability of neuroticism scores, are given by Kirk et al[27] and Birley et al.[29]

PHENOTYPES

The study participants completed a questionnaire that included a shortened Composite International Diagnostic Interview,[30] which provided *DSM-IV*[31] lifetime diagnoses of depression (code 296.2: major depressive disorder, single episode; code 296.3: major depressive disorder, recurrent episode; or code 300.4: dysthymic disorder) and anxiety (code 300.23: social phobia; code 300.02: generalized anxiety disorder; code 300.01: panic with agoraphobia; code 300.21: panic without agoraphobia; code 300.22: agoraphobia without panic; and code 300.03: obsessive-compulsive disorder). Standard clinical signifi-

Table 1. Description of Pedigrees Available for Initial Association Analysis

	DEPorANX	DEP	ANX	EPQ-20%	K10-20%
Total No. of pedigrees*	443	365	279	304	305
No. of individuals in pedigrees	1955	1620	1232	1360	1374
Total No. genotyped	1431	1183	894	991	1012
No. affected	624	486	352	384	382
No. affected with both parents genotyped	193	143	108	108	112
No. affected with 1 parent genotyped	145	111	86	98	97
No. of pedigrees ≥3 genotyped	288	239	179	200	205
No. of extended pedigrees†	3	0	1	3	2
No. of nuclear pedigrees with genotypes on:					
1 Affected only	18	39	49	234	239
1 Affected, ≥1 unaffected sibling‡	268	212	162	NA	NA
2 Affected siblings	83	62	35	67	59
2 Affected siblings, ≥1 unaffected‡	58	44	28	NA	NA
3 Affected siblings	12	4	5	5	9
3 Affected siblings, ≥1 unaffected‡	5	4	1	NA	NA
≥4 Affected siblings	0	0	0	1	0
4 Affected siblings, ≥1 unaffected‡	2	0	0	NA	NA

Abbreviations: ANX, any *DSM-IV* anxiety diagnosis; DEP, any *DSM-IV* depression diagnosis; DEPorANX, any *DSM-IV* depression or anxiety diagnosis; EPQ, Eysenck Personality Questionnaire; K10, 10-item Kessler Psychological Distress Scale; NA, not applicable.

*To be included in the analysis, each pedigree needed at least 1 affected genotyped individual and a genotyped parent or sibling.

†Most families were simple 2-generation nuclear families; extended pedigrees were either 3-generation families (grandparents, affected parents, and affected offspring) or 2-generation cousin families. Total number of nuclear families = total number of pedigrees plus extra nuclear families from extended families.

‡Unaffected siblings were not identified for EPQ-*x*% and K10-*x*%.

cance exclusion criteria[32] were applied, which helps to ensure accurate prevalence rates of *DSM-IV* diagnoses.[32] Prevalence rates in the sample compared with the Australian population are discussed by Kirk et al.[27] Diagnoses were coded as follows: 2 = affected, 1 = unaffected for all *DSM-IV* diagnoses, and 0 = not scored or affected for a different *DSM-IV* diagnosis. The phenotypes used for initial association analysis were DEP (any *DSM-IV* depression diagnosis), ANX (any *DSM-IV* anxiety diagnosis), and DEPorANX (any *DSM-IV* depression or anxiety diagnosis). Individual *DSM-IV* diagnoses that had more than approximately 100 affected participants were used to follow up on associations detected in the broad diagnosis groups.

The questionnaire completed by study participants also included the full 23-item EPQ-R neuroticism scale and the 10-item Kessler Psychological Distress Scale (K10),[33] a measure of current nonspecific psychological distress in the anxiety-depression spectrum. The EPQ-R and K10 scores were transformed to be residuals from regression of the arcsine transformed score[34] on age, sex, and age × sex. The distribution of the residuals was nonnormal, reflecting the population distribution of scores and the ascertainment criteria. For this reason and to facilitate comparison with the *DSM-IV* diagnostic criteria, individuals were considered to be "affected" for the EPQ-R and the K10 if their residual scores were in the top *x*th percentile of available residuals. Percentiles *x* = 5, 10, 15, and 20 were considered, and the resulting traits are labeled EPQ-*x*% and K10-*x*%.

In total, there were 2063 genotyped study participants from 990 families, with genotypes available on an additional 791 family members. A description of the families with at least 1 affected genotyped individual and with at least 1 parent or 1 sibling genotyped is given in **Table 1** for DEPorANX, DEP, ANX, EPQ-20%, and K10-20%. For example, there were 624 genotyped individuals with a diagnosis of anxiety and/or depression from 443 families. Numbers of affected individuals and pedigrees in common between phenotypes are given in **Table 2**. Given the overlap in definition of affected status between the phenotypic data sets, mutually exclusive phenotypic data subsets were created to follow up on initial association results.

SNP GENOTYPING

Mah et al[16] genotyped 67 SNPs in a 100-kb window around their most associated SNP (rs752016) in their discovery case-control sample and then selected 14 SNPs for genotyping on their case-control replication samples. Of these SNPs, 4 were selected for genotyping in their family-based replication samples (which included an Australian sample genotyped at the laboratory of the Queensland Institute of Medical Research). Two of these 4 SNPs were genotyped in the present sample (rs752016 and rs1327175). For better representation of the 50-kb region (**Figure**), rs841865 was replaced by 2 SNPs that bounded it (rs3736963 and rs2767565), and, similarly, rs2478813 and rs716461 were genotyped instead of rs249808. Genotyping was performed using primer extension on the MassARRAY system (Sequenom Inc) as described by James et al.[35] More than 99% of genotyped samples had genotypes for all 6 SNPs, with error rates less than 0.1%.[35]

STATISTICAL ANALYSIS

The Genetic Power Calculator[36] was used to investigate the power of the sample for a range of genetic model scenarios and assuming a transmission disequilibrium test design. A type I error of 0.05/30 = 0.0017 was chosen, conservatively assuming that we would perform independent tests with 6 SNPs × 5 phenotypes (considering the *x*% groupings of K10 and EPQ as single phenotypes because their results are interpreted as a set). For example, 300 nuclear families with 1 affected offspring have greater than 80% power to detect a genotyped causal locus of frequency 0.3, assuming a type I error of 0.0017 and a heterozygous genotype relative risk of 1.65 under a genetic model of multiplicative allelic action; 150 nuclear families could detect a causal genotyped locus with a heterozygous genotype relative risk of 2.00 using the same criteria. These calculations provide a baseline indication of power as the present study design includes many families with multiple affected siblings or unaffected siblings, which should result in increased power.[37] The software program SIB-PAIR[38] was used to check for departures from Hardy-Weinberg equilibrium

Table 2. Number of Affected Individuals in Common (Above Diagonal) and Number of Pedigrees in Common (Below Diagonal) Between Phenotypes*

	Initial Association Analysis Phenotypes					Follow-up Association Analysis Phenotypes								
									Major Depressive Disorder					
	DEPorANX	DEP	ANX	EPQ-20% (Neuroticism)	K10-20% (Psychological Distress)	ANXnotDEP	DEPnotANX	DEPandANX	Single (296.2)	Recurrent (296.3)	Generalized Anxiety Disorder (300.02)	Social Phobia (300.23)	Panic and/or Agoraphobia (300.01); Panic (300.21); Agoraphobia (300.22)	Obsessive-compulsive Disorder (300.3)
DEPorANX	624/443	486	352	277	248	138	272	214	257	218	140	126	99	106
DEP	365	486/365	214	211	190	0	272	214	257	218	104	78	56	55
ANX	279	201	352/279	197	170	138	0	214	111	97	140	126	99	106
EPQ-20%	245	211	184	384/304	240	66	80	131	102	103	75	81	50	73
K10-20%	238	206	168	214	382/305	58	78	112	89	96	71	73	38	60
ANXnot DEP†	130	52	130	78	68	138/130	0	0	0	0	36	48	43	51
DEPnot ANX†	235	235	71	121	123	28	272/235	0	146	121	0	0	0	0
DEPand ANX†	180	180	180	132	121	31	50	214/180	111	97	104	78	56	55
296.2	222	222	126	124	124	28	144	113	257/222	0	54	43	29	30
296.3	191	191	113	128	120	30	121	106	53	218/191	50	33	25	22
300.02	127	103	127	87	80	49	27	99	63	63	140/127	35	21	25
300.23	112	90	112	79	76	53	28	81	60	52	43	126/112	12	35
300.01 + 300.21 + 300.22	95	69	95	63	55	49	24	60	43	38	31	25	99/95	11
300.3	97	70	97	76	67	57	25	60	43	44	37	41	23	106/97

Abbreviations: ANX, any *DSM-IV* anxiety diagnosis; ANXnotDEP, any *DSM-IV* diagnosis for anxiety but not depression; DEP, any *DSM-IV* depression diagnosis; DEPandANX, *DSM-IV* diagnoses for both depression and anxiety; DEPnotANX, any *DSM-IV* diagnosis for depression but not anxiety; DEPorANX, any *DSM-IV* depression or anxiety diagnosis; EPQ, Eysenck Personality Questionnaire; K10, 10-item Kessler Psychological Distress Scale.

*Using as an example $x = 20$ for EPQ-20% and K10-20%. Diagonal cells contain the number of affected individuals/number of pedigrees for each phenotype. The number of pedigrees in common can exceed the number of affected individuals in common because different family members may be affected in each data set.

†The individuals considered affected for DEPorANX were separated into the mutually exclusive subgroups of DEPandANX, DEPnotANX, and ANXnotDEP.

and to estimate the $|D'|$ and r^2 measures of linkage disequilibrium from parental or inferred parental genotypes.

Association analysis was undertaken using 2 different methods: the pedigree disequilibrium test (PDT)[37] and the transmission disequilibrium score test (TDST).[39] The PDT calculates a measure of association within each family and then combines the measures for all families. It is a test for linkage disequilibrium (ie, linkage and association), and it uses information from extended families and from affected and unaffected individuals. In a linear logistic regression framework, the TDST uses information from 1 or more affected offspring from nuclear families only and constructs case and control haplotypes from all parental chromosomes. The test is equivalent to the haplotype-based haplotype relative risk test[40] when there is only 1 affected offspring per family and when all genotypes are known. As a within-family association test, the PDT is valid in the presence of population stratification and gains power from using both concordant and discordant siblings and extended families. In contrast, the TDST uses only affected offspring (Clayton[39] argued that the amount of information from unaffected offspring in this test would be negligible) and considers only nuclear families but gains power from being an association test across families and should also be robust to population stratification.[39] Because only affected offspring were identified for EPQ-*x*% and K10-*x*%, only TDST analysis was undertaken for these traits. Single- and 2-marker haplotype tests PDT and TDST were conducted as implemented in the software program UNPHASED[41] (*pdtphase* and *tdtphase*, respectively) with the options *missing*, to allow imputation of missing parental genotypes; *EM*, to allow estimation of uncertain haplotype frequencies; and *rare 0.01*, to require haplotypes to have a frequency greater than 1%. The option *condition* was used to investigate the independence of associated markers, and the option *sibsex* was used to investigate sex-specific associations. Permutation tests were performed when the asymptotic *P* value was less than .05, using the options *permutation 10 000* and, for TDST, *robustperm* to account for the family structure. The resulting empirical *P* values account for a nonasymptotic distribution of the test statistic resulting from the structure of the data. The *permutation 10 000* option used with the *window 2* and *individual* (TDST only) options provided an empirical *P* value for the most significant 2-marker haplotype correcting for the multiple testing of all 2-marker haplotypes. All pedigrees and genotypes were included for each analysis even when no individuals were measured or had affected family members so that all available genotypes were available for estimation of haplotype frequencies and determination of missing parental genotypes. The ORs representing the increased frequency of a haplotype in cases vs controls were calculated relative to the haplotype with the highest frequency from the frequencies estimated in TDST.

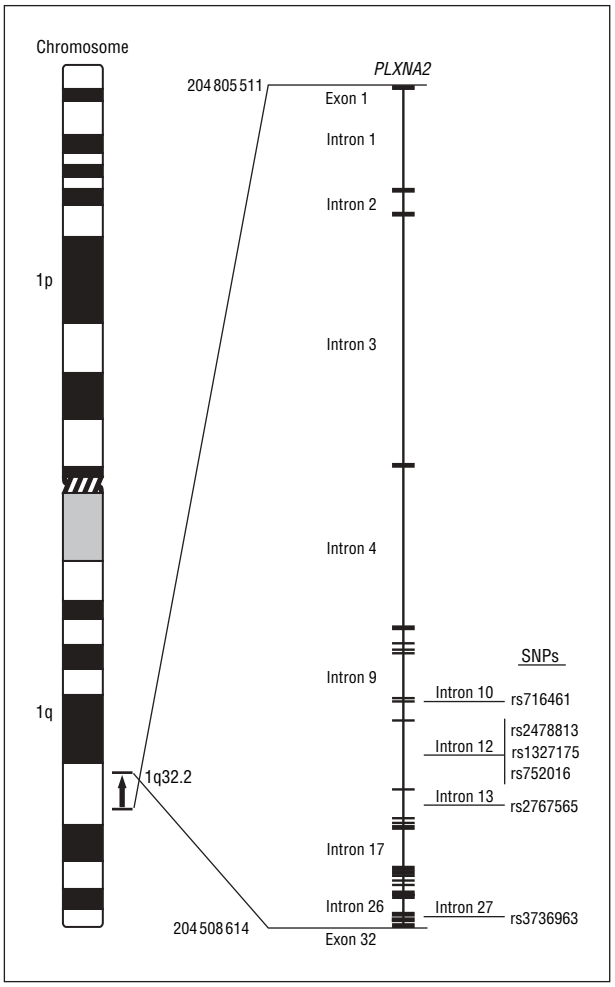

Figure. Chromosomal location and gene structure of the gene encoding plexin A2 (*PLXNA2*) and position of genotyped single nucleotide polymorphisms (SNPs). The arrow indicates that the orientation of the gene is inverted compared with that of the chromosome.

RESULTS

No SNPs showed evidence of departure from Hardy-Weinberg equilibrium. Minor allele frequencies, $|D'|$, and r^2 measures of linkage disequilibrium show high linkage disequilibrium on the $|D'|$ scale, but, as expected from the differences in allele frequencies, linkage disequilibrium is low on the r^2 scale (**Table 3**).

Association results for individual SNPs and DEPorANX, DEP, ANX, EPQ-*x*%, and K10-*x*% are listed in **Table 4**. Empirical *P* values (based on permutations for individuals tests) are reported when $P<.05$. In general, *P* values from permutation testing were in agreement with the asymptotic *P* values for PDT. Asymptotic *P* values for TDST were smaller than the corresponding empirical *P* values for TDST, presumably because the different family structures in this design cause the distribution of the test statistic to deviate from theoretical expectations. Empirical *P* values for PDT and TDST were mostly in good agreement to the same order of magnitude, suggesting that most information for association comes from affected offspring and that there is no evidence of population stratification. The following discussion and follow-up analyses consider TDST results only.

All 5 phenotypes (considering the *x*% groupings of K10 and EPQ as single phenotypes because the results are interpreted as a set) show evidence ($P<.05$) of association with the SNP rs2478813, and the association with ANX ($P<.001$) retains significance after Bonferroni correction ($P<.002$) for the 30 tests undertaken (it is also significant if we consider the *x*% groupings as individual tests, a total of 66 tests $P<.001$). The minor allele A of rs2478813 is associated with the affected status of the phenotypes. There is also evidence of associations between the SNPs rs3736963, rs2767565, and rs752016 and DEPorANX, ANX, and DEP, but there is little evidence of association between the genotyped SNPs immediately flanking rs2478813 (rs1327175 and rs716461). Although there may be more than 1 underlying associated variant, or an underlying associated variant that is present on the background of a haplotype of multiple genotyped SNPs, these results can all be explained by a single underlying associated variant that is most highly associated with the SNP rs2478813. First, rs2478813 shows a high $|D'|$ relationship with the other 5 SNPs but a moderate to low r^2 relationship (Table 2), which reflects the difference in allele frequencies between the SNPs and the absence (for $|D'|=1$ or near absence for high $|D'|$) of 1 of the 4 possible haplotypes, when considering the haplotypes of pairs of SNPs each including rs2478813. The minor allele A of the SNP rs2478813 is coupled predominantly with the common alleles C and G (Table 2) of the genotyped SNPs flanking rs1327175 and rs716461, respectively, so that the strong association between rs2478813 and the 5 traits does not result in any correlated associations with rs1327175 and rs716461. In contrast, the high $|D'|$ relationship between rs2478813 and the first 3 genotyped SNPs (rs3736963, rs2767565, and rs752016) reflects the coupling of the minor A allele of rs2478813 with the minor alleles of the other SNPs so that the strong association between the phenotypes and rs2478813 is reflected as weaker associations with rs3736963, rs2767565, and rs752016. Second, haplotype analysis of pairs of SNPs showed no association to be more significant than that with rs2478813 (results not shown). Last, conditional SNP association analysis of each of the first 3 SNPs, conditioned on the association with rs2478813, are all nonsignificant, suggesting that the observed SNP single associations all result from the same underlying variant. Further investigation and discussion focuses on the association with rs2478813 only.

The association between rs2478813 and the phenotype DEPorANX ($P=.007$) is found to be driven mostly by the association with ANX ($P<.001$), although a tendency toward association is seen for DEP alone ($P=.07$). The *DSM-IV* diagnoses of anxiety and depression from Composite International Diagnostic Interview questionnaires are not mutually exclusive (Table 2). Therefore, to provide insight into these results, we considered the mutually exclusive subgroups of DEPandANX (*DSM-IV* diagnoses for both depression and anxiety), DEPnotANX (any *DSM-IV* diagnosis for depression but not anxiety), and ANXnotDEP any *DSM-IV* diagnosis for anxiety but not depression). The numbers of affected individuals and

Table 3. Pairwise Measures of Linkage Disequilibrium and Minor Allele Frequencies of Genotyped SNPs

SNP	Pairwise Measures of Linkage Disequilibrium*						Alleles	MAF†	Distance From rs3736963, kb
	rs3736963	rs2767565	rs752016	rs1327175	rs2478813	rs716461			
rs3736963		0.94	0.79	0.50	0.90	0.33	G<A	0.42	0.0
rs2767565	0.26		0.80	0.67	0.79	0.95	A<G	0.18	22.3
rs752016	0.19	0.62		0.99	0.99	0.99	G<A	0.18	29.8
rs1327175	0.02	0.14	0.30		0.89	0.99	G<C	0.06	39.3
rs2478813	0.16	0.42	0.63	0.01		0.99	A<G	0.12	39.4
rs716461	0.06	0.07	0.08	0.05	0.05		A<G	0.28	49.8

Abbreviations: kb, kilobase; MAF, minor allele frequency; SNP, single nucleotide polymorphism.
*$|D'|$ above the diagonal and r^2 below the diagonal.
†Corresponds to the first listed allele.

Table 4. Association Analysis Between Individual SNPs and the 5 Initially Considered Phenotypes*

Phenotype	Analysis Method	SNPs					
		rs3736963	rs2767565	rs752016	rs1327175	rs2478813	rs716461
DEPorANX	PDT	.03	.01	.02	.33	.01	.55
	TDST	.04	.007	.01	.56	.007	.4
DEP	PDT	.09	.03	.05	.39	.03	.87
	TDST	.10	.05	.08	.71	.07	.37
ANX	PDT	.03	.006	.007	.79	<.001	.92
	TDST	.06	.01	.02	.29	<.001	.43
Neuroticism							
EPQ-5%	TDST	.67	.68	.18	.33	.06	.25
EPQ-10%	TDST	.45	.2	.16	.34	.01	.49
EPQ-15%	TDST	.64	.35	.38	.16	.04	.65
EPQ-20%	TDST	.39	.33	.25	.11	.01	.34
Psychological distress							
K10-5%	TDST	.08	.14	.15	.44	.03	.62
K10-10%	TDST	.38	.4	.43	.03	.01	.68
K10-15%	TDST	.47	.25	.35	.02	.01	.68
K10-20%	TDST	.25	.27	.48	.04	.03	.85

Abbreviations: ANX, any *DSM-IV* anxiety diagnosis; DEP, any *DSM-IV* depression diagnosis; DEPorANX, any *DSM-IV* depression or anxiety diagnosis; EPQ, Eysenck Personality Questionnaire; K10, 10-item Kessler Psychological Distress Scale; PDT, pedigree disequilibrium test; SNPs, single nucleotide polymorphisms; TDST, transmission disequilibrium score test.
*Asymptotic *P* values are given when $P>.05$, and empirical *P* values calculated from 10 000 permutations are given when $P<.05$.

pedigrees for these data sets are listed in Table 2. By reducing the number of affected individuals analyzed, the association between SNP and affectation status must increase to retain the same level of significance. However, evidence of association was found for DEPandANX ($P=.004$) and ANXnotDEP ($P=.009$) but not for DEPnotANX ($P=.93$) (**Table 5**). Further understanding of the associations can be gained by considering association analysis of the major subdiagnoses that contribute to the ANXandDEP diagnoses (Table 5). All the anxiety diagnoses (generalized anxiety disorder, social phobia, agoraphobia and panic disorder, and obsessive-compulsive disorder) show excessive transmission of allele A to affected offspring (OR, 2.8, 5.4, 2.3, and 1.9, respectively) and some evidence of association with rs2478813 ($P=.005$, .003, .07, and .13, respectively). In contrast, of the depression diagnoses, single-episode major depressive disorder (code 296.2) shows evidence of association ($P=.02$) but recurrent major depressive disorder (code 296.3) does not ($P>.99$). Only 111 individuals are comorbid for code 296.2 and ANX. Separate analysis of code 296.2andANX (OR, 3.0; $P=.01$) and 296.2notANX (OR, 1.5; $P=.30$) showed that individuals who are affected for both anxiety and depression are contributing most to the association of code 296.2 with the SNP rs2478813. In contrast, the 97 individuals who are comorbid for 296.3andANX (remembering that diagnoses 296.2 and 296.3 are mutually exclusive) show no significant association with rs2478813 ($P=.11$), although the minor allele is overtransmitted to affected offspring (OR, 2.1). Differences in age at interview, age at onset of depression, and sex in individuals affected with 296.2andANX vs 296.3andANX provided no further insight into these results. Analysis of the sexes separately for ANX showed no evidence of sex specificity of the association (124 males affected, $P=.047$; 228 females affected, $P<.001$; difference, $P=.25$).

The personality measures of EPQ neuroticism and K10 showed evidence of association with rs2478813. As the *x* of EPQ-*x*% and K10-*x*% increases, 2 conflicting forces

Table 5. TDST Association Analysis of Phenotypes With the SNP rs2478813*

	Affected Individuals, No.	*P* Value	Frequency, %†		OR (95% CI)‡
			Cases	Controls	
Initial Association Analysis					
DEPorANX	624	.007	14	9	1.6 (1.3-2.1)
DEP	486	.07	14	10	1.5 (1.1-1.9)
ANX	352	<.001	15	6	2.7 (1.9-3.8)
EPQ-5%	96	.06	15	5	3.1 (1.5-6.3)
EPQ-10%	194	.01	14	6	2.6 (1.6-4.2)
EPQ-15%	292	.04	13	8	1.7 (1.2-2.4)
EPQ-20%	384	.01	13	8	1.8 (1.3-2.5)
K10-5%	93	.03	11	3	4.3 (1.7-10.8)
K10-10%	189	.01	14	5	2.8 (1.7-4.7)
K10-15%	284	.01	13	7	2.1 (1.4-3.2)
K10-20%	382	.03	13	8	1.7 (1.2-2.4)
Follow-up Association Analysis					
ANXnotDEP§	138	.009	15	6	2.6 (1.5-4.6)
DEPnotANX§	272	.93	13	13	1.0 (0.7-2.0)
DEPandANX§	214	.004	15	6	2.7 (1.7-4.2)
296.2 (MDD single)	257	.02	16	9	1.9 (1.3-2.7)
296.3 (MDD recurrent)	218	>.99	12	12	1.0 (0.7-1.5)
300.02 (Generalized anxiety disorder)	140	.005	18	7	2.8 (1.7-4.7)
300.23 (Social phobia)	126	.003	14	3	5.4 (2.5-11.6)
300.01 + 300.21 + 300.22 (Panic and/or agoraphobia)	99	.072	14	6	2.3 (1.2-4.5)
300.3 (Obsessive-compulsive disorder)	106	.13	13	7	1.9 (1.0-3.6)
296.2andANX	111	.01	15	5	3.0 (1.5-5.8)
296.2notANX	146	.25	16	12	1.5 (0.9-2.3)
296.3andANX	97	.11	15	8	2.1 (1.1-4.0)
296.3notANX	121	.14	9	14	0.6 (0.3-1.0)
K10-15%andANX	143	.002	15	3	5.8 (2.7-12.0)
K10-15%notANX	141	.54	12	10	1.2 (0.7-2.1)

Abbreviations: ANX, any *DSM-IV* anxiety diagnosis; ANXnotDEP, any *DSM-IV* diagnosis for anxiety but not depression; CI, confidence interval; DEP, any *DSM-IV* depression diagnosis; DEPandANX, *DSM-IV* diagnoses for both depression and anxiety; DEPnotANX, any *DSM-IV* diagnosis for depression but not anxiety; DEPorANX, any *DSM-IV* depression or anxiety diagnosis; EPQ, Eysenck Personality Questionnaire; K10, 10-item Kessler Psychological Distress Scale; MDD, major depressive disorder; OR, odds ratio; SNP, single nucleotide polymorphism; TDST, transmission disequilibrium score test.

*Asymptotic *P* values are given when *P*>.05, and empirical *P* values from 10 000 permutations are given when *P*<.05.

†Frequency of the minor allele A in cases (transmitted to affected offspring) and controls (not transmitted to affected offspring).

‡Expresses the increased risk of allele A being transmitted to affected offspring compared with allele G.

§The individuals considered affected for DEPorANX were separated into the mutually exclusive subgroups of DEPandANX, DEPnotANX, and ANXnotDEP.

affecting the power of association are changed. Phenotypic homogeneity decreases (reflected in the trend toward decreasing ORs) (Table 5) as *x* increases, but the sample size increase results in lower confidence intervals for the OR (Table 5). As *x* increases, the proportion of individuals considered affected for the EPQ-*x*% and the K10-*x*% who are also affected for ANX decreases (0.71, 0.61, 0.56, and 0.51 for the EPQ and 0.67, 0.57, 0.50, and 0.44 for the K10). An example analysis of K10-15% (the most significantly associated) as K10-15%andANX and K10-15%notANX suggests that it is the anxiety personalities that are driving the associations with the personality measures (*P*=.002 and *P*=.54, respectively).

COMMENT

In this study we tested the hypothesis that a gene found to be associated with schizophrenia might also be associated with other psychiatric and psychological conditions. The gene *PLXNA2* encodes for plexin 2A, which acts as a receptor for the class 3 semaphorins,[14] which as well as showing widespread expression during neuronal development are persistently expressed in the adult nervous system,[8] are involved in neuronal apoptosis,[10] and have been shown to induce pruning of hippocampal axon branches in the adult brain.[11] The present analysis found evidence of an association between rs2478813 (and other SNPs correlated with it) and anxiety, depression, neuroticism, and psychological distress. The association with anxiety can be declared significant after stringent Bonferroni correction for multiple testing. There is no evidence of multiple associated variants or of a 2-SNP haplotype of the genotyped SNPs being more highly associated with any of the phenotypes than rs2478813. The association reported by Mah et al[16] was for the SNP rs752016, but it was the major allele that was found to have higher frequency in cases than controls. This SNP is in high $|D'|$ linkage disequilibrium (0.99) and moderate r^2 (0.63) with rs2478813, and the minor alleles at both loci tend to be coupled. We found associations for the minor allele of the SNP rs752016 and anxiety and depression, but further investigation suggests that it reflects the same association as with rs2478813, weakened by the incomplete association between the SNPs.

Mah et al[16] reported an OR ranging from 1.31 to 1.49 for 5 of their discovery and replication samples that showed association with the major allele of the SNP rs752016. Only their Australian replication sample showed association (nonsignificant) with the minor allele. Mah et al[16] sequenced exons 10 to 15 in the 40-kb region around the 2 SNPs for 18 affected individuals but found no nonsynonymous SNPs and speculated that a causal variant may be mediated by variations that affect RNA splicing or expression levels. Only further studies will clarify whether the present findings and those of Mah et al[16] are true positives and determine whether common or different causal variants underlie these associations.

The initial association analysis phenotypes tested a specific hypothesis. However, the unique nature of the phenotyped study sample is that all participants completed the Composite International Diagnostic Interview, the EPQ, and the K10 so that all individuals were scored for all phenotypes. This allowed us to investigate further the phenotypic subtypes involved in the association. Such analyses, although a true reflection of the information provided by the present study sample, should be seen as hypothesis generating, and interpretation should proceed with caution until the results have been replicated in an independent sample. Association analysis of mutually exclusive diagnostic subgroups suggested that the association of rs2478813 with depression, neuroticism, and psychological distress was being driven by individuals who were comorbid for anxiety. Analysis of the major subclasses of anxiety all showed evidence of association despite their smaller sample sizes (although some comorbidity exists between subtypes). A genetic linkage study[42] of anxiety susceptibility found evidence of linkage to 1q ($P = .04$), a region chosen for study because of its homology to a murine chromosomal region (chromosome 1, 56-106 cM) found to contain quantitative trait loci for anxiety.[43] Henderson et al,[44] in a genomewide linkage study of behavioral traits in mice, also reported strong evidence (logarithm of odds >20) for a dominant quantitative trait locus on chromosome arm 1q for generalized anxiety in response to relatively safe stimuli as opposed to anxiety resulting from highly anxiogenic stimuli. The powerful design of their study resulted in a tight 95% confidence interval (71-78 cM) for the position of the quantitative trait locus. The mouse ortholog of *PLXNA2* is located in this interval (http://www.informatics.jax.org/searches/linkmap_form.shtml), which strengthens the argument that variants of *PLXNA2* have a causal role in anxiety disorders.

Semaphorin receptors are plausible candidates for a common etiology of a spectrum of psychiatric and psychological disorders because of their role in the development, maintenance, and apoptosis of the nervous system.[8,9] Semaphorins were discovered only 13 years ago, and the more than 20 semaphorin genes that are known to date are highly conserved from invertebrates to humans.[9] Understanding of the role of plexins acting together with neuropilins as receptors for semaphorins is also relatively new.[12] The first genomewide association study of Parkinson disease was recently published,[45] and the most highly associated SNP was in the gene encoding semaphorin 5A. The present results contribute to the growing body of evidence that semaphorins and semaphorin receptors may have an important role in a wide range of neurologic and psychiatric disorders. From association results it is not possible to determine whether the putative functional role of *PLXNA2* operates in neurodevelopment or adult neurogenesis. Nonetheless, these results are the first to provide evidence of a specific genetic variant that could contribute to the waning and waxing of adult neurogenesis that has been hypothesized[3] to precipitate the onset of and recovery from depression. Suboptimal performance of adult neurogenesis in anxiety disorders has not previously been proposed, but these results suggest that this area merits further investigation.

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