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Evidence for Linkage and Association of *GABRB3* and *GABRA5* to Panic Disorder

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Panic disorder (PD) is a debilitating anxiety disorder characterized by episodes of intense fear with autonomic and psychological symptoms that lead to behavioral impairment. A convergence of genetic and biological evidence implicates gamma-aminobutyric acid type A receptor subunits on chromosome 15q12 as candidate genes for PD. This study investigated 120 Caucasian, multiplex PD pedigrees using regional microsatellites (chr15q11–13) and found support for linkage (logarithm of odds (LOD) $\geqslant 2$), with a prominent parent-of-origin effect. Genotyping with 10 single-nucleotide polymorphisms (SNPs) showed linkage to *GABRB3* (rs11631421, LOD = 4.6) and *GABRA5* (rs2075716, LOD = 2.2), and allelic association to *GABRB3* (rs8024564, $p = 0.005$; rs8025575, $p = 0.02$) and *GABRA5* (rs35399885, $p = 0.05$). Genotyping of an independent Sardinian PD trio sample also supported association in the region, again with a parent-of-origin effect. These findings provide genetic evidence for the involvement of the genes *GABRB3* and *GABRA5* in the susceptibility to PD.

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INTRODUCTION

Panic disorder (PD) is an anxiety disorder characterized by paroxysmal panic attacks and impairment from the ensuing anticipatory anxiety about recurring attacks. Panic attacks manifest with intense anxiety symptoms such as pounding heart, difficulty breathing, sweating, dizziness, fear of dying, and an urge to flee or escape (Marquez *et al*, 2001; Starcevic *et al*, 1993). The lifetime prevalence of PD, including those with and without agoraphobia, is 4.8–5.1% in the United States (Kessler *et al*, 2006; Grant *et al*, 2006), with a 1.9–3.5-fold higher prevalence in females, depending upon age (Eaton *et al*, 1994). The estimated relative risk to siblings of PD probands is 5- to 10-fold higher than the population risk, and the heritablity of PD is 0.48 (Hettema *et al*, 2001), making PD amenable to gene-mapping efforts. Segregation studies suggest a complex genetic etiology that reflects phenotypic and locus heterogeneity. In a genome scan of 120 Caucasian multiplex pedigrees, we observed evidence for a susceptibility locus for PD on chromosome 15q12 (D15S822, two-point heterogeneity logarithm of odds (HLOD) = 2.0) (Fyer *et al*, 2006). The highest LOD-transformed non-parametric linkage score (NPL-LOD), 2.56, occurred close to the centromere on chromosome 15 and defined a peak of ~12 cM (1.1–13.6 cM) in which NPL-LOD scores were consistently >2.0. We carried out empirical significance testing, and the 15q region achieved an empirical *p*-value of 0.04 for the model-specific analysis. This region is notable for a high density of segmental duplications, the imprinted Prader–Willi/Angelman Syndrome region, and a cluster of GABA receptor subunit genes. In this study, we carried out further mapping of the 15q region in the same PD sample to narrow the region of linkage by fine-mapping with microsatellites, single-nucleotide polymorphisms (SNPs) at *GABRB3* and *GABRA5*, followed by mutation screening and parent-of-origin transmission effects in probands by a separate analysis of a Sardinian PD sample. To characterize potential baseline functional variation related to genetic involvement of this region, we also tested genetic effects on familial expression patterns of implicated GABR genes in an unphenotyped sample.

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MATERIALS AND METHODS

Subject Recruitment and Sample Collection

A total of 1591 individuals (992 DNA samples) from 120 Caucasian, mulitplex PD pedigrees of primarily Western European descent were recruited from anxiety clinics, therapists, and support groups from the Northeastern United States. Subjects were assessed with the Lifetime Version of the Schedule for Affective Disorders and Schizophrenia (SADS-LA) by blinded clinician interviewers, as described elsewhere (Fyer and Weissman, 1999). A detailed narrative summary for each relative who had panic symptoms was prepared, as was a checklist of medical conditions (including age of onset). At least two senior clinical investigators (AJF, MMW, and Donald F Klein) independently reviewed the case material and diagnosed each individual as to level of affectedness for PD and age of onset. Six levels of PD diagnosis were defined as follows: definite, probable, possible, or any panic, and unaffected, or unknown. These were used to establish three phenotypic designations for statistical analysis such as narrow (definite or probable PD), intermediate (adding possible PD), and broad (adding any panic) PD phenotypes (Hamilton *et al*, 1999). All subjects provided informed consent, and the experimental protocol was approved by the institutional review boards of the University of California, San Francisco and the New York State Psychiatric Institute. Genomic DNA was isolated from peripheral blood or lymphoblastoid cell lines as described elsewhere (Knowles *et al*, 1998).

Sardinian Families

Trios (PD patients and their biological parents) were recruited from the Department of Psychiatry of the University of Sassari and at the Psychiatric Unit of the General Hospital of Nuoro. Probands were assessed with the SADS-L-Anxiety Version. AR, who had been previously trained in this instrument by our group, supervised translation and back translation of the instrument into Italian and of the case narratives from Italian to English; conducted training sessions with the interviewers in Sardinia to establish diagnostic reliability and teach narrative writing techniques; and carried out ongoing monitoring through review of narratives and site visits to assure reliability and validity of interviews. Case narratives were reviewed for diagnostic consistency by AJF. Cases considered questionable were additionally reviewed by MMW, followed by discussion between the two reviewers to reach a consensus. A total of 49 families (147 individuals) were clinically evaluated with the SADS-L-Anxiety Version and provided DNA samples for this study. Probands were sequentially consenting patients with PD at the two Sardinian clinical sites. The 147 individuals include: 39 parent/child triads ($n = 117$), six proband/parent/sibling triads ($n = 18$), five sibling pairs ($n = 10$), and one proband/father pair ($n = 2$).

Genotyping by Fragment Analysis and 5′ Nuclease Assay

Microsatellite markers were chosen to cover $\sim$7.14 Mb ($\sim$19 cM) on chromosome 15q11.2-q13.1. We used seven known markers from extant linkage mapping panels, and developed one additional novel polymorphic marker more centromeric than available microsatellites on the long arm of the acrocentric chromosome 15. The average inter-marker spacing was 1.02 Mb and $\sim$2.73 cM. Genotyping details are described in the Supplementary Methods.

Resequencing Analysis of GABRB3 and GABRA5 in PD Probands

We performed *GABRA5* and *GABRB3* mutation screening in a subset of 92 PD probands from the larger set of PD pedigrees, using standard procedures, as described in Supplementary Methods.

SNP Genotyping in Sardinian PD Families

In the Sardinian PD trios, we used the Illumina GoldenGate assay to type $\sim$335 evenly spaced SNPs in a 5.34-Mb region within the 15q region described above. DNA samples from 139 individuals in 49 families, consisting of 34 trios and 15 families of other configurations, were genotyped. Data were output in the form of intensity files and analyzed using Illumina's BEADSTUDIO software. Our final data set included 316 SNPs and 135 samples (97%) with a mean per sample call rate of 99.7% and a mean per SNP call rate of 99.8%. Details are provided in the Supplementary Methods. Given the difference in selection processes for each of the genotyping components, there was no overlap of SNP markers between the linkage SNPs and the Sardinian fine-mapping SNPs. We thus genotyped the SNPs from the linkage mapping of US pedigrees in the Sardinian sample.

Linkage and Association Analysis in US PD Pedigrees

Linkage analysis was performed using several methods corresponding to different underlying biological models for 8 regional microsatellites and 10 evenly spaced SNPs across the genomic region encompassing *GABRB3* and *GABRA5*. First, multipoint LOD-transformed NPL scores were computed, measuring identity-by-descent allele-sharing among affected family members, using GENEHUNTER (Kruglyak *et al*, 1996). Second, a two-point parametric linkage analysis was performed. Maximum LOD scores for genetic linkage were reported, maximized over mode of inheritance (dominant/recessive) (Greenberg *et al*, 1998; Hodge *et al*, 1997), recombination fraction, and the proportion of linked pedigrees under the assumption of locus heterogeneity (HLODs), as is commonly performed in genetic linkage analysis. Parametric assumptions in the models were based upon previous segregation analyses (Vieland *et al*, 1996), which showed that dominant and recessive single major locus models are equally predictive of the mode of transmission for PD. All LOD scores were computed assuming a 1% phenocopy rate and 50% penetrance. Risk allele frequencies under dominant and recessive genetic models were set to and 1% and 20%, respectively. Based upon sexually dimorphic prevalence rates in PD, unknown or unaffected phenotypes were assigned 66% and 33% penetrance, and 14% and 7% phenocopy rate in females and males, respectively. Parametric LOD scores were generated using FASTLINK (Cottingham *et al*, 1993; Schaffer *et al*,

1994), and HLODs were computed using HOMOG. A three-point (genomic position and two flanking markers) parametric linkage analysis incorporating a parent-of-origin effect was computed using LINKAGE-IMPRINT (Shete and Zhou, 2005). For this analysis, the penetrance was set to 50% and the risk allele frequency was set to 4%. HLODs for the parent-of-origin analysis were computed directly from the by-pedigree LOD scores using R (Ihaka and Gentleman, 1996).

Allelic association analysis in nuclear trios and extended pedigrees was determined using the family-based association test (FBAT) (Laird *et al*, 2000; Horvath *et al*, 2001). Multimarker haplotypes were tested for association using FBAT. We report uncorrected *p*-values, owing to the prior evidence for involvement of the 15q region in PD, although we used permutation to assess empirical significance in selected analyses. Association and parent-of-origin analysis of genotypic data in the Sardinian subset of families was carried out using PLINK (Purcell *et al*, 2007) and UNPHASED (Dudbridge, 2003).

Parametric linkage, NPL, and allelic association scores were determined for broad, intermediate, and narrow phenotypic definitions for PD. Parent-of-origin linkage analysis was performed using the broad phenotypic definition only.

Genetics of Candidate Gene Expression in CEPH Trios

We investigated the genetics of *GABRA5* and *GABRB3* gene RNA expression patterns in cell lines from 30 uncharacterized Caucasian child–parent trios ($n = 90$) of European ancestry from Utah (Coriell Institute for Medical Research, Camden, NJ), for whom genotypic annotation is publicly available via the HapMap Consortium Project (Release 20/phase II Jan 2006 on NCBI B35 assembly, dbSNP b125, www.hapmap.org). Genotypes for non-redundant SNPs (ie, excluding tagging proxy SNPs at correlation coefficient of $r^2 = 1.0$) with minor allele frequencies (MAFs) $\geqslant 0.05$ were tested for association with expression levels using the quantitative transmission disequilibrium test using UNPHASED (Dudbridge, 2003). Details on cell culture conditions and gene expression analysis are described in the Supplementary Methods.

RESULTS

Our previous work found evidence for linkage between PD and a region of chromosome 15q (Fyer *et al*, 2006), localized near D15S822, in the proximity of the *GABRB3* and *GABRA5* genes. We first interrogated the implicated region in chr15q11–13 with eight microsatellites to further refine the linkage interval in the same PD pedigree sample. Several of the eight microsatellites showed suggestive linkage (LOD $\geqslant 2$) using two-point analyses under the assumption of heterogeneity. For example, under a recessive model, the two markers, *GABRB3* and D15S156, separated by 1.2 Mb, showed HLODs ~ 2 using broad, intermediate, or narrow definitions of PD (Figure 1). Multipoint analysis largely supported this finding, with a maximal NPL score of 2.93 (NPL-LOD = 1.86, $p = 0.004$) under the broad PD diagnostic model at the *GABRB3* microsatellite marker. Multipoint parent-of-origin analysis was carried out, and evidence for linkage was found in the region with maximal maternal HLOD = 3.06, and a maximum paternal HLOD = 1.60, with both scores maximizing at different locations in the interval (Supplementary Figure 1). Given the positional findings, in addition to plausibility as biological candidates, we elected to pursue our findings by additional genotyping using 10 SNPs in the vicinity of the *GABRB3* and the neighboring *GABRA5* candidate gene. For two-point parametric analysis, we observed linkage to SNPs in *GABRB3* (rs11631421, HLOD = 4.56), as shown in Figure 2 under a recessive model and a broad diagnostic model. For a dominant model, the highest score was found in *GABRA5* (rs2075716, HLOD = 2.22), also with a broad diagnostic model (data not shown). Non-parametric multipoint analysis lead to a peak NPL score of 3.70 (NPL-LOD = 2.97, $p = 0.0008$) occurring under a broad diagnostic model on the *GABRA5* SNP rs140682, a synonymous SNP occurring in a valine residue conserved among mammals (data not shown).

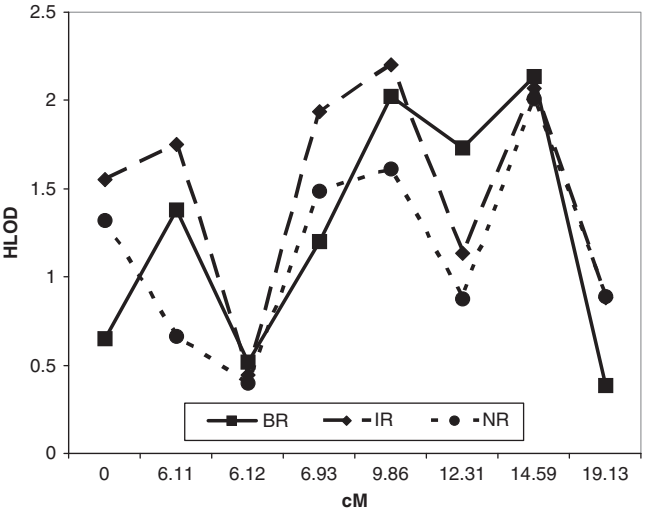

Figure 1 Fine-mapping of chr15q11–13 in 120 multiplex, Caucasian panic disorder (PD) pedigrees by two-point linkage, given locus heterogeneity. HLOD = heterogeneity logarithm of odds; diagnostic phenotypes are: B = broad, I = intermediate, and N = narrow PD; R = recessive mode of inheritance; cM = centiMorgan.

Nominal allelic association was found for SNPs at *GABRB3* (rs8025575, $p = 0.005$; rs8024564, $p = 0.02$) and *GABRA5* (rs35399885, $p = 0.05$) under a broad diagnostic scheme (Figure 3). Haplotypic analysis did not markedly improve the association signal for the two associated *GABRB3* SNPs ($p = 0.03$).

Sardinian Sample

To further explore association to PD in the chromosome 15q region, we genotyped 34 trios from Sardinia composed of a proband with PD and biological parents. We genotyped 316 markers across a 5-Mb region centered on the GABA receptor subunit genes. Using the transmission disequilibrium test (Spielman *et al*, 1993), the marker showing the greatest evidence for association was rs10220768 ($p = 0.003$), a SNP occurring in a relatively gene-poor region ~ 400 kb upstream of the necdin gene (*NDN*) (Supplementary Figure 2). The next best marker was rs2017247, a SNP lying in the 3′ untranslated region (UTR) of the

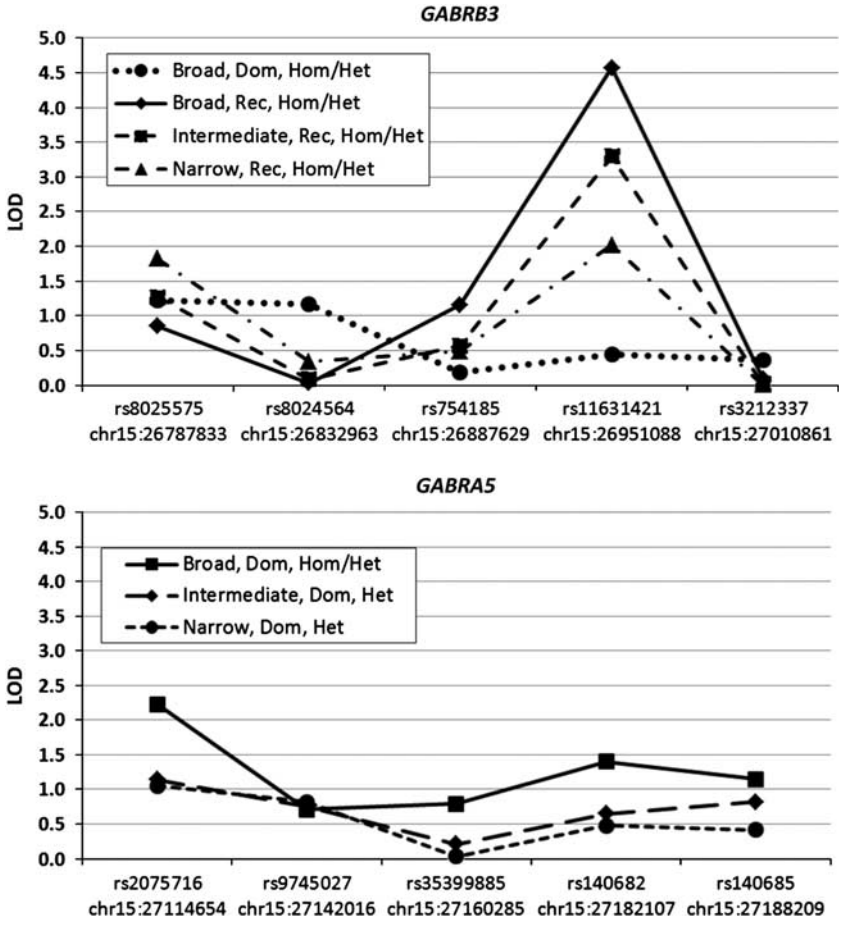

Figure 2 Two-point linkage scores for five single-nucleotide polymorphisms (SNPs) in each of the *GABRB3* (upper panel) and *GABRA5* (lower panel) genes in 120 multiplex, Caucasian panic disorder (PD) pedigrees from the US logarithm of odds (LOD); SNPs are shown in genomic context as listed in Supplementary Table 1. Diagnostic phenotypes are broad, intermediate, and narrow PD; Het = locus heterogeneity; Hom = locus homogeneity; Dom = dominant mode of inheritance; Rec = recessive mode of inheritance; variant position per Feb 2009 (hg19).

GABRB3 gene ($p = 0.004$). This marker was completely correlated with rs2912586, 5.4 kb downstream of *GABRB3*. The permuted pointwise empirical *p*-value for this pair of markers was 0.008 (Table 1). When an additional 15 families were added (mostly discordant sibling pairs with a single biological parent) and the data were analyzed with UNPHASED, the results remained essentially identical. Among the 10 SNPs originally genotyped in our North American PD pedigrees, two *GABRB3* markers showed *p*-values <0.1 in Sardinian PD trios (rs3212337, $p = 0.03$; rs11631421, $p = 0.07$). Given the parent-of-origin effect seen in the linkage analysis from the US pedigrees, and the known complex imprinting status of genes throughout the 15q11–13 region, we considered transmissions from heterozygous parents separately to estimate a parent-of-origin effect in the Sardinian trios. When assessing paternal parent-of-origin effect, the best marker was rs2017247 ($p = 0.002$). The pointwise permuted *p*-value was 0.0009. Of 13 heterozygous fathers, 12 transmitted the risk allele and only one did not (Supplementary Figure 3). Conversely, for 11 heterozygous mothers, the ratio was 7 to 4 ($p = 0.82$). This marker was also associated with PD in the entire trio set, as described above. The maternal parent-of-origin analysis yielded a best marker with a *p*-value of 0.007 (rs1045935), with a pointwise permuted *p*-value of 0.004. This SNP occurs in an open reading frame for a primate-specific transcript expressed in brain that is downstream of the *SNRPN/SNURF* gene complex. The ratio of maternal transmission:non-transmission was 16:4, compared with 9:9 for paternal transmission. The best paternal and maternal findings are almost 1.5 Mb apart.

Mutation Screen in US PD Probands

A mutation screen of *GABRB3* and *GABRA5* in a subset of 92 PD probands revealed 9 novel *GABRB3* variants (Supplementary Table 4) and 14 novel *GABRA5* variants (Supplementary Table 5) of predominantly uncommon (<0.05) MAFs in introns. However, two novel *GABRB3* variants in the 3′ UTR of the gene may potentially alter the predicted GATA and NCX (or TLX2 and HOX11L1) transcription factor binding sites (TFBSs). One additional novel *GABRB3* indel at chr15:26866266 was detected at high MAF (0.50). In *GABRA5*, novel variants were observed in the 3′ UTR and 5′ UTR at low MAF. Among the known variants observed in our PD probands, most showed no significant deviation in allele frequency from the publicly available databases for Caucasians except *GABRA5* rs41309256 C>T, with MAF of 0.03 in 60 Caucasians according to dbSNP, and 0.13 in PD cases.

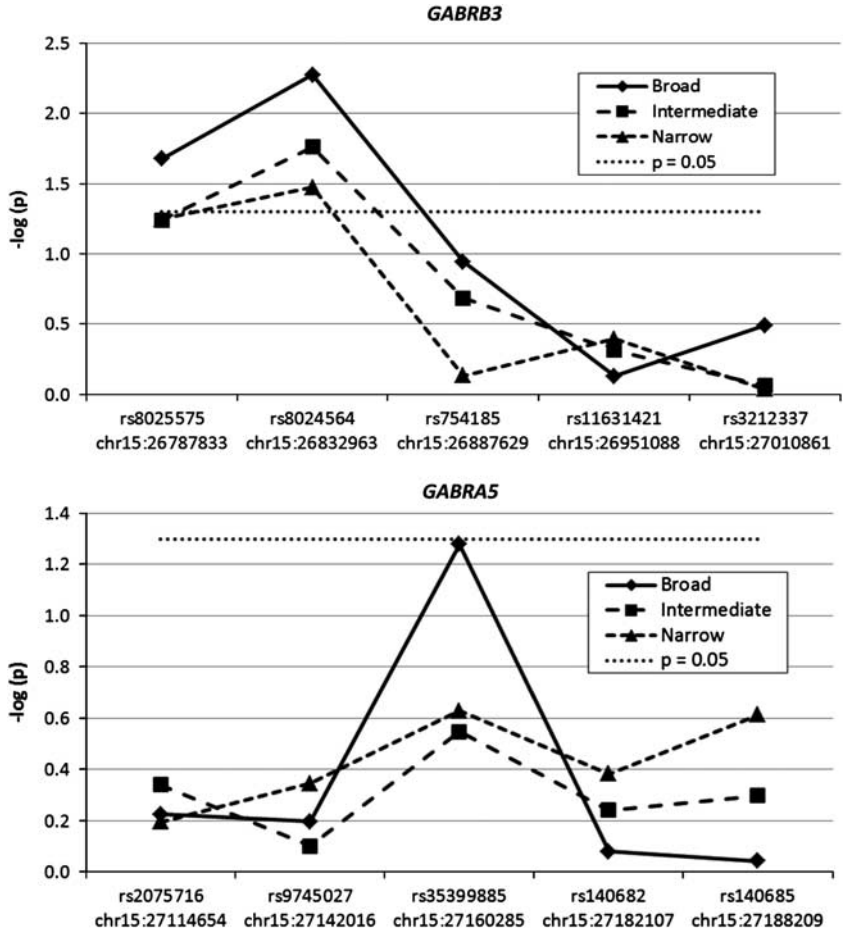

Figure 3 Association analysis for five single-nucleotide polymorphisms (SNPs) in each of the *GABRB3* (upper panel) and *GABRA5* (lower panel) genes in 120 multiplex, Caucasian panic disorder (PD) pedigrees from the United States. The *p*-values obtained from family-based association test (FBAT) are depicted by transforming by taking the negative log of the *p*-value. The dotted line depicts a nominal *p*-value of 0.05. Diagnostic phenotypes are broad, intermediate, and narrow PD; variant position per Feb 2009 (hg19).

Table 1 Allelic Association to SNPs in a 5.3-Mb Region Surrounding *GABRB3* in 34 Sardinian PD Trios

SNP	Position, Feb 2009 (hg19)	*p*(TDT)	OR (95% CI)	*p*(emp)
rs10220768	chr15:24319693	0.002	0.27 (0.11, 0.67)	0.008
rs2912586	chr15:26783311	0.004	3.80 (1.42, 10.18)	0.008
rs2017247	chr15:26789162	0.004	3.80 (1.42, 10.18)	0.008
rs8029900	chr15:24867594	0.007	4.00 (1.34, 11.96)	0.015
rs12914251	chr15:24889850	0.007	4.00 (1.34, 11.96)	0.015
rs1382058	chr15:27758904	0.009	0.38 (0.17, 0.81)	0.01
rs4778471	chr15:22964125	0.009	3.17 (1.27, 7.93)	0.018

Abbreviations: OR (95% CI), odds ratio of TDT with 95% confidence interval; PD, panic disorder; *p*(EMP), pointwise empirical *p*-value after 1 million permutations; *p*(TDT), *p*-value of TDT; SNP, single-nucleotide polymorphism. Position, location at the UCSC genome browser (hg19, build GRCHr37). Results are shown for SNPs with *p*(TDT) <0.01. The SNPs rs2912586 and rs2017247 show an $r^2 = 1.0$, as rs8029900 and rs12914251.

Genetics of GABRB3 Expression in CEPH Trios

Out of $>23\,000$ RNA transcripts interrogated on the BeadChip, 6255 were detected in all 90 CEPH cell line samples. *GABRA5* expression was below the limit of detection, but *GABRB3* was reliably detected in 35 samples derived from 20 unrelated individuals, six parent–child dyads, and one trio. Four of 198 tested SNPs spanning the *GABRB3* locus were associated with significantly altered *GABRB3* RNA expression levels (rs2315904, rs10519566, rs7183628, and rs6576613) after 10 000 permutations ($p \leqslant 0.01$) (Table 2). None of these four SNPs were genotyped in our PD samples. The most significantly associated SNP, rs2315904, exhibited a *p*-value of 0.0002 (permutation $p = 0.0001$) and met strict correction for 198 tests. This SNP lies in an intron of *GABRB3*.

DISCUSSION

Chr15q and GABRB3 Variant Association to PD

In this study, we sought to further characterize the linkage between chromosome 15q and PD that we observed in our Caucasian sample of 120 multiplex PD pedigrees (Fyer *et al*, 2006). We found additional support for association between the 15q region and PD, despite the very small size of the Sardinian trio sample. When we tested the same 10 SNPs used in the US cohort again in the Sardinian sample, we saw repeated association to *GABRB3* SNP rs3212337 in both

Table 2 Genotypic Association with *GABRB3* Expression in Lymphoblastoid Cell Lines from 35 Unphenotyped Caucasians

SNP	Position, Feb 2009 (hg19)	Location	Permuted *p*-value	SNPs captured $r^2 = 1.0$; MAF = 0.05
rs2315904	chr15:26967522	GABRB3 intron	1.0 E–04	rs2315903
rs10519566	chr15:24526963	GABRB3 intron	0.0006	NA
rs7183628	chr15:26995727	GABRB3 intron	0.008	NA
rs6576613	chr15:27034566	5′ of GABRB3	0.006	rs12595843, rs7497801

Abbreviations: MAF, minor allele frequencies; SNP, single-nucleotide polymorphism.

samples. As in many multistage gene-mapping projects, we have used different SNP markers in different samples, which might have implications for interpretation of results. Our use of different markers for the same targeted region in different samples can be explained by the principles governing genetic analysis in the kind of populations we used, and given the aims we sought to achieve. The use of 8 microsatellites and 10 SNPs targeted to candidate PD genes within the implicated region parsimoniously fulfilled our aim to more precisely pinpoint the linkage signal seen in 120 multiplex, US pedigrees from our previously published work. By their nature, pedigrees contain high linkage disequilibrium in the range with the targeted area that fewer variants were needed capture a signal in this type of sample. However, our aim to corroborate this finding in trios required a greater number of markers as compensation for reduced information and linkage disequilibrium in this type of sample. We genotyped the same 10 fine-mapping SNPs from the US pedigrees in the Sardinian sample that were genotyped for over 300 additional SNPs. In some ways, different markers at the same candidate gene region sufficiently reinforces the hypothesis of the involvement of *GABR* genes in PD. To illustrate the point further, because so many genetic markers are harbored together in long haplotypes, linkage and association to a given SNP often does not reveal the 'causative' allele. This is a natural limitation of genetic analysis at this scale. Ultimately, future functional studies would be needed to show evidence that a given allele is causal for PD.

Parent-of-Origin Effects

In both our US and Sardinian samples, we found evidence of parent-of-origin effects. Interestingly, we observed both maternal and paternal parent-of-origin effects that were not overlapping, and consistent with the documented sources of complex pattern of imprinting in this chromosomal region.

GABRB3 and GABRA5 Mutation Screening in PD

A mutation screen of the *GABRB3* and *GABRA5* genes found no amino-acid changes but a number of novel synonymous coding variants in both genes in this subset of PD probands. *In vitro* experiments will be required to determine whether the SNPs discovered in the 3′ and 5′ UTRs of *GABRA5* have functional roles, such as translational regulation by altering codon usage or transcriptional regulation by binding sites for enhancers or supressors of expression.

Genetic Association with GABRB3 Expression in CEPH Trios

To understand possible endophenotypes that may inform putative GABAergic dysregulation in PD, we sought to characterize the expression of the GABA receptor subunit genes in the uncharacterized Caucasian subset of HapMap samples. We performed a test of genetic association with expression levels as a quantitative trait in lymphoblastoid cell lines from CEPH (Centre d'Étude du Polymorphisme Humaine) (Dausset *et al*, 1990) CEU trios to look for genotypic association with gene expression. We found three intronic SNPs and one SNP lying 5′ to *GABRB3*, in the intergenic region between the 5′ ends of *GABRB3* and *GABRA5*, which were associated with *GABRB3* expression in lymphoblastoid cell lines. Perfect linkage disequilibrium ($r^2 = 1.0$) between the associated rs6576613 variant and untested SNPs (rs12595843 and rs7497801) broaden the implicated region to ~1180 bp, which is separated by another 1200 bp from the nearest cluster of predicted TFBSs (Sp-1, NF-YA, and NF-YB). Characterization of genetically mediated GABAergic signaling patterns in PD is needed. Recent magnetic resonance spectroscopy by Long *et al* (2013) showed significantly lower cortical GABA levels in PD patients than controls, with more exaggerated deficits among patients with a family history of PD.

Continued Support for GABAergic Dysregulation in PD

The biological role for *GABR* genes in the pathogenesis of PD is supported by mechanistic, ethological, and clinical evidence. They control neuronal excitability and reduce anxiety via binding to ligands such as benzodiazepines (BZs) used as short-term treatment for PD, as well as to endogenous GABA and neurosteroids. *GABRB3* deletion, as in Angelman Syndrome (Holopainen *et al*, 2001) and knockout mice (Sinkkonen *et al*, 2003), leads to neurobehavioral effects (DeLorey *et al*, 1998; DeLorey *et al*, 2008) and reduced BZ binding. *GABRA5* mediates ligand selectivity and affinity (Strakhova *et al*, 2000) and possibly sedation (van Rijnsoever *et al*, 2004). PD patients exhibit altered GABA levels (Goddard *et al*, 2004) and lower BZ binding in the brain (Cameron *et al*, 2007; Bremner *et al*, 2000). Panic attacks in PD subjects yield lower than normal levels of neurosteroids (Strohle *et al*, 2003); whereas PD medications effectively raise central GABA levels (Parent *et al*, 2002;

Sanacora *et al*, 2002; Bhagwagar *et al*, 2004). Pleiotropic effects of GABAergic dysregulation may explain syndromic features of PD (Weissman *et al*, 2000; 2004), such as migraine, a common comorbidity also linked to chr15q12 and treated with GABAergic drugs (Russo *et al*, 2005). Non-equal chromosomal expression (Gimelbrant *et al*, 2007) or allele-specific expression of disease genes (Tanaka *et al*, 2012; Pernhorst *et al*, 2011) such as parent-of-origin expression differences in expression (Kong *et al*, 2009) can be pathological. For example, maternal overtransmission of the Ser11 allele of *GABRB3* rs25409 (C87T, Pro11Ser) results in three- to sixfold greater relative risk for autism (Delahanty *et al*, 2011). Our data support the hypothesis that genetic variation in the 15q region, perhaps at *GABRB3* and *GABRA5*, is related to risk for PD and may involve allele-specific expression. However, other studies showed that *GABRB3* and *GABRA5* were not associated with PD in a study of 26 pedigrees from the United States and Iceland (Crowe *et al*, 1997); and linkage analysis (Gelernter *et al*, 2001; Crowe *et al*, 2001; Gregersen *et al*, 2012) and genome-wide association studies (Erhardt *et al*, 2012; Otowa *et al*, 2012) failed to implicate this region. We view these findings as exploratory, although evidence in more than one PD sample suggests that *GABRB3* and *GABRA5* are good candidates to explain at least a portion of the biological and genetic factors in PD pathology.

Parent-of-Origin Findings

These findings are intriguing, as genes in the chromosome 15q11–13 region are subject to alelle-specific imprinting and large-scale deletions of the non-imprinted maternal or paternal alleles result in Angelman/Prader–Willi syndromes, respectively. The *GABRB3* and *GABRA5* genes are largely reported to be biallelically expressed, although there are reports of uniparental expression (Meguro *et al*, 1997) in cell systems. Parent-of-origin effects have been documented with *GABRB3* at the gene expression level in autism (Hogart *et al*, 2007), as well as at the genotypic level with alcohol dependence (Song *et al*, 2003). In a subset of our current US PD pedigree collection, we observed nominal parent-of-origin effects for the cumulative lifetime risk for PD when examining patterns of transmission, and noted that the ratio of female to male affected offspring was higher when the disease appeared to be maternally transmitted (Haghighi *et al*, 1999). No parent-of-origin effects were observed in a smaller number of Italian pedigrees with PD and agoraphobia (Battaglia *et al*, 1999). However, in our Sardinian trios, we observed parent-of-origin effects that appear to be linked to different areas of chromosome 15, according to either maternal or paternal transmission of PD.

In summary, we found evidence for association and linkage between PD and the region of the *GABRB3* and *GABRA5* genes in both United States and Sardinian Caucasian families, and a number of novel sequence variants in the region of these two genes among PD probands. In addition, we found support for the genetic contribution to *GABRB3* expression in Caucasians, which suggests a possible regulatory mechanism for the hypothesized GABAergic dysregulation in PD. Future studies could further develop these findings with more comprehensive linkage disequilibrium mapping, with additional replication data, and with large-scale sequence analysis across the entire region.

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