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Association of the *GABRD* Gene and Childhood-Onset Mood Disorders

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Abstract

The chromosome 1p36 region was previously indicated as a locus for susceptibility to recurrent major depressive disorder based on a linkage study in a sample of 497 sib pairs. We investigated the GABA_A δ receptor subunit gene, *GABRD*, as a susceptibility gene to childhood-onset mood disorders (COMD) because of substantial evidence implicating GABAergic dysfunction in mood disorders and the position of this gene near the 1p36 linkage region. Using a sample consisting of 645 Hungarian families with a child/adolescent proband diagnosed with a mood disorder with the onset of the first episode before age 15, we found some evidence for association of two polymorphisms located within the gene, rs2376805 and rs2376803, as well as significant evidence for biased transmission of the haplotypes of these two markers (global χ^2 test for haplotypes = 12.746, 3df, $p=0.0052$). Further, significant evidence of association was only observed in male subjects ($n=438$) when the results were analyzed by sex ($\chi^2=9.000$, 1df, $p=0.003$ for rs2376805). This was in contrast with the previous linkage findings, as LOD scores exceeding 3 were only in female-female pairs in that study. These findings point to the *GABRD* gene as a susceptibility gene for COMD, however, this gene may not explain the previous linkage finding.

Keywords

GABRD; child; depression; genetics; mood disorders; GABA; GABA receptors; chromosome 1p

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Introduction

Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the mammalian brain, has been implicated in the pathogenesis of mood disorders based on several lines of evidence including low cerebrospinal fluid and plasma levels of GABA, and more recently proton magnetic resonance spectroscopy indicating low GABA concentrations in the occipital cortical and prefrontal regions (Berrettini et al., 1983, Petty et al., 1993, Sanacora et al., 2004, Krystal et al., 2002, Brambilla et al., 2003, Tunnicliff and Malatynska, 2003, Hasler et al., 2007). The GABA_A receptors (GABA_ARs) are constructed from a large number of possible subunits with different subunit compositions providing different physiological and pharmacological functions which contribute to the heterogeneous roles of GABA_A receptors in the mammalian brain (Mohler, 2006, Jacob et al., 2008). The majority of GABA_A receptors are composed of two α subunits, two β , and one γ or δ subunit. Those composed of $\alpha 4$ or $\alpha 6$ subunits together with β and δ subunits are predominantly located at extrasynaptic sites and mediate tonic inhibition in the brain (Mohler, 2006). The δ subunit is highly expressed in brain and expression has been reported in cerebellum, cerebral cortex, medulla, occipital pole, frontal lobe, temporal lobe and putamen (Windpassinger et al., 2002). Of the GABA receptors, the δ subunit-containing GABA_A receptors are particularly implicated in mood disorders. These receptors have a high affinity for GABA and are the preferential targets of neuroactive steroids (Stell et al., 2003), which are endogenous steroids (progesterone, pregnenolone, 3 α , 5 α -tetrahydroprogesterone, dehydroepiandrosterone, dehydroepiandrosterone sulfate) implicated in anxiety and depression (Eser et al., 2006, Longone et al., 2008).

Genetic studies also implicate the GABAergic system in mood disorders. The recently published Wellcome Trust Case Control Consortium (WTCCC) genome-wide association study for bipolar disorder found strong evidence that genetic variation in the GABA_A receptor $\beta 1$ subunit gene, *GABRB1*, influences risk for RDC schizoaffective disorder, bipolar type (Craddock et al., 2010). Further study of this specific clinical subtype in that sample identified association of the *GABRA4*, *GABRB3*, *GABRA5* and *GABRR1* genes. Previous to that study, a number of the GABA subunit genes had been investigated in linkage and association studies of unipolar and bipolar disorder with conflicting results (Brambilla et al., 2003). Sample size may be a factor in the conflicting findings for some of the studies, particularly for early studies.

The δ subunit encoded by the *GABRD* gene is a positional candidate, as well as a functional candidate based on the position of this gene in 1p36, a region with suggestive evidence for linkage with recurrent major depressive disorder (McGuffin et al., 2005). Association studies of the *GABRD* gene have not been reported to date. There were no genotyped markers in the *GABRD* gene in the WTCCC bipolar association study (Craddock et al., 2010), likely due to the small size of the gene (~11 kb), thus they were unable to test this gene in that sample.

Based on the biologic function of GABAergic systems in the pathogenesis of mood disorders, particularly the special role of the δ subunit in GABA_A receptors and the linkage findings pointing to the 1p36 region, we investigated the role of the *GABRD* gene in childhood-onset mood disorders using a large family-based sample from Hungary. Our sample consisted of 645 nuclear families (807 affected children) with a proband diagnosed with a mood disorder with the onset of the first episode by age 15.

Subjects

The diagnostic assessment, inclusion/exclusion criteria and clinical characteristics of the families enrolled in this study have been published previously (Liu et al., 2006, Kiss et al., 2007, Kapornai et al., 2007, Tamas et al., 2007, Baji et al., 2009). Families were recruited from 23 mental health facilities across Hungary as part of a multidisciplinary Program Project to study risk factors in childhood-onset mood disorders (Liu et al., 2006). The probands were diagnosed using the Interview Schedule for Children and Adolescents - Diagnostic Version (ISCA-D), an extension and modification of the ISCA (Sherrill and Kovacs, 2000). Children and parents were interviewed separately and approximately one month later both were interviewed again by a different clinician (psychologist/psychiatrist). Children were interviewed about themselves and parents about their child's symptoms. The final diagnosis was based on consensus among two independent child psychiatrists. The probands met DSM-IV criteria of a mood disorder (depressive or bipolar disorder), with the onset of the first episode by 14.9 years of age. For 14 of the siblings, who also were affected, onset of a mood disorder was between 15 and 18 years of age and they were diagnosed using the same procedure as with probands. This study met with IRB approval at the University of Pittsburgh, the Centre for Addiction and Mental Health, Toronto, and all recruitment centers in Hungary. Written informed consent was obtained from parent and child.

The analyses for this study were based on the genotypes of 645 families with 807 affected children (438 males, 369 females). At the time of diagnosis, 0.8% of the children met criteria for bipolar disorder. Given the young age of the children, we expect that 20 to 30% of those currently diagnosed with unipolar depression will develop bipolar disorder as they mature. Because it is not possible to predict which of these children will develop bipolar disorder, they cannot be excluded from our sample.

Genotyping

DNA was extracted from blood using a high salt method (Miller et al., 1988). The polymorphisms were genotyped using the TaqMan® 5' nuclease assay with primers and probes available commercially (Applied Biosystems, Foster, CA, TaqMan® SNP Genotyping Assay). Five µl PCR reactions contained 30 ng of genomic DNA, 2.5 µl of 2 × TaqMan® Universal PCR Master Mix (Applied Biosystems) and 0.125 µl of 40 × SNP genotyping assay mix which contained 36 µM of each primer and 8 µM of each probe. The thermal cycling conditions were 95°C for 10 min, then 40 cycles of 95°C for 15 seconds and 1 minute at the annealing temperature. Two negative controls were included for each 96 well plate. Plates were read on the ABI 7900-HT Sequence Detection System using the allelic discrimination end-point analysis mode of the software package version 2.0 (Applied Biosystems).

Statistical Analysis

All data were screened for Mendelian errors using PEDSTATS, and MERLIN to detect any crossovers between markers (Abecasis et al., 2002). This data set was free of any detectable Mendelian errors and none of the markers used deviated from Hardy-Weinberg Equilibrium (Table 1).

The TDTPHASE program of the UNPHASED package was used to test for biased transmission of alleles (Dudbridge, 2003). Association of haplotypes was evaluated using TRANSMIT with the robust estimator option (Clayton and Jones, 1999). Haplotypes with frequencies less than 10% were pooled and χ^2 and p values are only reported for those with frequencies greater than 10%. The degree of linkage disequilibrium (LD) between markers

was evaluated using Haploview v3.2 (<http://www.broad.mit.edu/mpg/haploview>) (Barrett et al., 2005) (Table 2).

Results

Since few polymorphisms have been validated in the *GABRD* gene, there were no tag SNP markers identified within the gene in HapMap that could be used for this study. The 4 polymorphic SNPs in HapMap, all located in intron 1 had the same minor allele frequency, indicating strong linkage disequilibrium (LD). We selected four informative SNP markers across the gene to genotype, two were within the gene region (rs2376805 located in intron 1 and rs2376803 3' to the gene, 5.8 kb from the coding region), one was located at the far 5' side (rs3795283, 34 kb from the coding region) and the other was located 3' to the gene (rs7513222, 66 kb from the coding region). We further genotyped a reported, but not validated, non-synonymous SNP rs28753776 (Thr161Ser) on the sample but did not find any evidence for variation in the DNA of 188 individuals tested.

The single marker TDT analysis for the four polymorphic markers is shown in Table 1. The two SNPs within the gene region exhibited biased transmission of alleles to the affected offspring, rs2376805 and rs2376803. Permutation analysis was performed on the TDT analysis. The best p value ($p=0.012$) however was no longer significant after correcting for the number of markers ($p=0.062$).

We examined the LD between the markers (Table 2) and observed LD only between the two markers within the *GABRD* gene region (rs2376805 and rs2376803). We performed haplotype analysis using these two markers and observed significant biased transmission of haplotypes to the affected children. One common haplotype with frequency 68.5% was over-transmitted to affected offspring ($\chi^2=11.495$, 1df, $p=0.0007$) while another common haplotype with frequency 17.5% was less often transmitted to the affected children ($\chi^2=8.16$, 1df, $p=0.0043$), with a global value of $\chi^2=12.746$, 3df, $p=0.0052$ (Table 3).

As the previous linkage study only observed evidence for linkage on chromosome 1p36 in female-female pairs (McGuffin et al., 2005), we stratified our sample by sex and examined the transmissions to female ($n=369$) and male ($n=438$) children separately for the analysis of single markers (Table 4) and haplotypes (Tables 5 and 6). Significant evidence of association was only observed in male subjects (Tables 4 and 5). This contrasts to the findings from the linkage study that found evidence for linkage only in females (McGuffin et al., 2005).

Discussion

In this study we investigated the *GABRD* gene based on substantial evidence for GABA dysfunction in mood disorders and the position of the gene near the region linked with recurrent depressive disorders. We found evidence for association between genetic markers in the *GABRD* gene and COMD, with haplotypes significantly biased in transmission. Further, the association appeared to be sex specific; being restricted to the males in the sample. These results, particularly, the significant results from haplotype analyses, indicate that genetic variation in the *GABRD* gene could contribute to the linkage signal in the 1p36 region reported in a previous genome scan for recurrent depressive disorders. However, the results were only significant in males, in contrast to the previous linkage findings.

While sex appears to be a factor in the association of *GABRD* in our study and the previous linkage study, it's not clear why the findings are in opposite directions. This discrepancy could be explained in the linkage study by the small number of male-male pairs (58 compared to 266 female pairs) with low power on their own to detect an effect. However, it

does not explain the LOD score in the female pairs (3.03 in females compared to 1.9 in the entire sample and -0.08 in males).

Phenotype may also be a factor in the discrepant findings. Our sample was composed of childhood and adolescent probands with the onset of the first episode by 15 years old (mean age of first onset 10.8 years, S.D. 2.28). There is some evidence that earlier age of onset may have unique genetic risks. A recent large twin study of depressive and anxiety symptoms in children and adolescents at ages 8-9, 13-14, 16-17, and 19-20 supports a common genetic factor across these ages as well as unique genetic contributions at each age point (Kendler et al., 2008). Both genetic innovation (new genes that were previously without effect on the phenotype become active) and attenuation (genes that impact at one developmental age that decline in their influence during subsequent periods) were supported across these age groups. Although not significant, there was also evidence that the genetic correlation between males and females decreased with increased age, indicating the possibility of sex specific differences in genetic risk at different ages. Thus, age, and sex related specific genetic risks should be considered as a possible explanation. Alternatively, the association in *GABRD* detected here may not be contributing to the linkage signal. That paper describes the peak location as 13.8 to 21.8 cM corresponding to ~7.2 to 14.5 Mb on Build 35. This would be greater than 5 Mb centromeric to *GABRD* located at 1.99 Mb. Studies of this gene in the linkage sample of families would be optimal and replication in independent samples is of course required before any conclusions can be made.

Interestingly, there are some data indicating a role of the δ receptor in pubertal anxiety in females (Shen et al., 2007). The neurosteroid allopregnanolone (TPH), released in response to stress, has been shown to increase anxiety like behavior in pubertal mice in contrast to an anxiety-reducing effect in pre-pubertal and adult mice (Shen et al., 2007). Studies indicate that the effects of TPH reverse from enhancing GABA-gated current at $\alpha 4\beta 2\delta$ receptors to inhibiting current in a Cl^- -dependent manner. Specifically implicating the δ subunit was the finding of up to two fold increased expression of this subunit in the CA1 hippocampus region of mice at the onset of puberty. Thus sex and developmental influences on the expression of this gene may contribute to the finding of association only in males in this age group.

Twin and family studies indicate that mood disorders are complex, with multiple genes involved, and any one gene will be unlikely to have a large contribution to risk. Association studies now point to a few candidate genes with support from multiple studies (Craddock and Forty, 2006). Here we report evidence for the *GABRD* gene as associated with childhood-onset mood disorders. Our finding, in conjunction with the recent reported association findings for the GABA subunit genes reported in the whole genome associations studies, indicate a substantial role of GABA dysfunction in the pathogenesis of mood disorders. DNA variants in the *GABRD* gene could modify gene expression or function and hence, affect the subunit composition and the physiological and pharmacological properties of the GABA_A receptors. Further work should address the functional DNA changes in the gene and investigate the mechanisms behind the observed sex specific effect.

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References

Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet.* 2002; 30:97-101. [PubMed: 11731797]

- Baji I, Lopez-Duran NL, Kovacs M, George CJ, Mayer L, Kapornai K, Kiss E, Gadoros J, Vetro A. Age and sex analyses of somatic complaints and symptom presentation of childhood depression in a Hungarian clinical sample. *J Clin Psychiatry*. 2009; 70:1467–72. [PubMed: 19709501]
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21:263–5. [PubMed: 15297300]
- Berrettini WH, Nurnberger JI Jr, Hare TA, Simmons-Alling S, Gershon ES, Post RM. Reduced plasma and CSF gamma-aminobutyric acid in affective illness: effect of lithium carbonate. *Biol Psychiatry*. 1983; 18:185–94. [PubMed: 6403063]
- Brambilla P, Perez J, Barale F, Schettini G, Soares JC. GABAergic dysfunction in mood disorders. *Mol Psychiatry*. 2003; 8:721–37. 715. [PubMed: 12888801]
- Clayton D, Jones H. Transmission/disequilibrium tests for extended marker haplotypes. *Am J Hum Genet*. 1999; 65:1161–9. [PubMed: 10486335]
- Craddock N, Forty L. Genetics of affective (mood) disorders. *Eur J Hum Genet*. 2006; 14:660–8. [PubMed: 16721402]
- Craddock N, Jones L, Jones IR, Kirov G, Green EK, Grozeva D, Moskvina V, Nikolov I, Hamshere ML, Vukcevic D, Caesar S, Gordon-Smith K, Fraser C, Russell E, Norton N, Breen G, St Clair D, Collier DA, Young AH, Ferrier IN, Farmer A, McGuffin P, Holmans PA, Donnelly P, Owen MJ, O'donovan MC. Strong genetic evidence for a selective influence of GABA(A) receptors on a component of the bipolar disorder phenotype. *Mol Psychiatry*. 2010; 15:146–53. [PubMed: 19078961]
- Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol*. 2003; 25:115–21. [PubMed: 12916020]
- Eser D, Romeo E, Baghai TC, Di Michele F, Schule C, Pasini A, Zwanzger P, Padberg F, Rupprecht R. Neuroactive steroids as modulators of depression and anxiety. *Neuroscience*. 2006; 138:1041–8. [PubMed: 16310959]
- Hasler G, Van Der Veen JW, Tuminis T, Meyers N, Shen J, Drevets WC. Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Arch Gen Psychiatry*. 2007; 64:193–200. [PubMed: 17283286]
- Jacob TC, Moss SJ, Jurd R. GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nat Rev Neurosci*. 2008; 9:331–43. [PubMed: 18382465]
- Kapornai K, Gentzler AL, Tepper P, Kiss E, Mayer L, Tamas Z, Kovacs M, Vetro A. Early developmental characteristics and features of major depressive disorder among child psychiatric patients in Hungary. *J Affect Disord*. 2007; 100:91–101. [PubMed: 17113651]
- Kendler KS, Gardner CO, Lichtenstein P. A developmental twin study of symptoms of anxiety and depression: evidence for genetic innovation and attenuation. *Psychol Med*. 2008; 38:1567–75. [PubMed: 18578897]
- Kiss E, Gentzler AM, George C, Kapornai K, Tamas Z, Kovacs M, Vetro A. Factors influencing mother-child reports of depressive symptoms and agreement among clinically referred depressed youngsters in Hungary. *J Affect Disord*. 2007; 100:143–51. [PubMed: 17125844]
- Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, Epperson CN, Goddard A, Mason GF. Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. *Mol Psychiatry*. 2002; 7(Suppl 1):S71–80. [PubMed: 11986998]
- Liu X, Gentzler AL, Tepper P, Kiss E, Kothencne VO, Tamas Z, Vetro A, Kovacs M. Clinical features of depressed children and adolescents with various forms of suicidality. *J Clin Psychiatry*. 2006; 67:1442–50. [PubMed: 17017833]
- Longone P, Rupprecht R, Manieri GA, Bernardi G, Romeo E, Pasini A. The complex roles of neurosteroids in depression and anxiety disorders. *Neurochem Int*. 2008; 52:596–601. [PubMed: 17996986]
- McGuffin P, Knight J, Breen G, Brewster S, Boyd PR, Craddock N, Gill M, Korszun A, Maier W, Middleton L, Mors O, Owen MJ, Perry J, Preisig M, Reich T, Rice J, Rietschel M, Jones L, Sham P, Farmer AE. Whole genome linkage scan of recurrent depressive disorder from the depression network study. *Hum Mol Genet*. 2005; 14:3337–45. [PubMed: 16203746]

- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988; 16:1215. [PubMed: 3344216]
- Mohler H. GABA(A) receptor diversity and pharmacology. *Cell Tissue Res.* 2006; 326:505–16. [PubMed: 16937111]
- Petty F, Kramer GL, Fulton M, Moeller FG, Rush AJ. Low plasma GABA is a trait-like marker for bipolar illness. *Neuropsychopharmacology.* 1993; 9:125–32. [PubMed: 8216695]
- Sanacora G, Gueorguieva R, Epperson CN, Wu YT, Appel M, Rothman DL, Krystal JH, Mason GF. Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. *Arch Gen Psychiatry.* 2004; 61:705–13. [PubMed: 15237082]
- Shen H, Gong QH, Aoki C, Yuan M, Ruderman Y, Dattilo M, Williams K, Smith SS. Reversal of neurosteroid effects at alpha4beta2delta GABAA receptors triggers anxiety at puberty. *Nat Neurosci.* 2007; 10:469–77. [PubMed: 17351635]
- Sherrill JT, Kovacs M. Interview schedule for children and adolescents (ISCA). *J Am Acad Child Adolesc Psychiatry.* 2000; 39:67–75. [PubMed: 10638069]
- Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABAA receptors. *Proc Natl Acad Sci U S A.* 2003; 100:14439–44. [PubMed: 14623958]
- Tamas Z, Kovacs M, Gentzler AL, Tepper P, Gadoros J, Kiss E, Kapornai K, Vetro A. The relations of temperament and emotion self-regulation with suicidal behaviors in a clinical sample of depressed children in Hungary. *J Abnorm Child Psychol.* 2007; 35:640–52. [PubMed: 17530394]
- Tunnicliff G, Malatynska E. Central GABAergic systems and depressive illness. *Neurochem Res.* 2003; 28:965–76. [PubMed: 12718451]
- Windpassinger C, Kroisel PM, Wagner K, Petek E. The human gamma-aminobutyric acid A receptor delta (GABRD) gene: molecular characterisation and tissue-specific expression. *Gene.* 2002; 292:25–31. [PubMed: 12119096]

Table 1

TDT results for single marker analyses

SNP	ABI assay ID	Allele	HWpval	Freq.	Trans	Not Transmitted	χ^2	p value (ldf)
rs3795283		A	1.00	0.333	231	252	0.913	0.339
rs2376805	C_16000020_10	G	0.33	0.189	147	193	6.243	0.012 ^a
rs2376803	C_16000021_10	C	0.47	0.312	208	258	5.375	0.020
rs7513222	C_30490003_10	A	0.43	0.348	242	250	0.130	0.718

^a 1000 permutations were performed using UNPHASED on the best *p* value to correct for the number of tests (global significance: *p*=0.062; SE=0.008). HWpval indicates the *p* value for the test of Hardy-Weinberg Equilibrium.

Table 2

Linkage Disequilibrium between Markers

	rs3795283	rs2376805	rs2376803	rs7513222
rs3795283		0.159	0.036	0.101
rs2376805	0.003		0.915	0.076
rs2376803	0.001	0.434		0.077
rs7513222	0.003	0.003	0.001	

Upper right shows the degree of linkage disequilibrium between markers as measured by D' and the bottom left as measured by r^2

Table 3

Haplotype analysis of the two markers in LD

rs2376805	rs2376803	Frequency	Observed	Expected	Var(O-E)	χ^2	p value (1df)
A	C	0.131	183.39	193.27	81.01	1.203	0.2727
G	C	0.175	234.60	262.97	98.67	8.160	0.0043
A	T	0.685	1073.60	1032.30	148.68	11.495	0.0007
G	T	0.009					

Global chisquared test for haplotypes with frequencies greater than 10% = 12.746, 3df, $p=0.0052$

TDT Analyses by Sex of the Child

	rs2376805 (G allele)					rs2376803 (C allele)				
	Freq	Trans	Not Trans	χ^2	p value (1df)	Freq	Trans	Not Trans	χ^2	p value (1df)
females	0.190	70	74	0.111	0.739	0.314	96	100	0.082	0.775
males	0.183	77	119	9.000	0.003	0.305	112	158	7.837	0.005

Table 5

Haplotype Analyses for Males

rs2376805	rs2376803	Frequency	Observed	Expected	Var(O-E)	χ^2	p value (1df)
A	C	0.124	95.12	101.08	43.91	0.808	0.3687
G	C	0.173	116.88	141.82	55.33	11.243	0.0008
A	T	0.696	595.88	563.74	81.29	12.711	0.0004
G	T	0.006					

Global chisquared test for haplotypes with frequencies greater than 10% = 14.517, 3df, $p=0.0022$

Table 6

Haplotype Analyses for Females

rs2376805	rs2376803	Frequency	Observed	Expected	Var(O-E)	χ^2	p value (1df)
A	C	0.133	79.21	85.75	34.82	1.229	0.2676
G	C	0.167	109.79	111.83	40.05	0.104	0.7471
A	T	0.686	465.79	455.68	64.09	1.595	0.2066
G	T	0.013					

Global chisquared test for haplotypes with frequencies greater than 10% = 2.215, 3df, p=0.5290