Molecular Psychiatry (2005) 10, 470–478

www.nature.com/mp

ORIGINAL RESEARCH ARTICLE

Sex-specific association between bipolar affective disorder in women and *GPR50*, an X-linked orphan G protein-coupled receptor

PA Thomson*[,1], NR Wray[1], AM Thomson[2], DR Dunbar[2], MA Grassie[2], A Condie[1], MT Walker[3], DJ Smith[3], DJ Pulford[2], W Muir[3], DHR Blackwood[3] and DJ Porteous[1]

[1]*Medical Genetics Section, University of Edinburgh, Molecular Medicine Centre, Western General Hospital, Crewe Road, Edinburgh, Scotland;* [2]*Organon Laboratories Ltd, Newhouse, Lanarkshire, Scotland;* [3]*Department of Psychiatry, Royal Edinburgh Hospital, Morningside Park, Edinburgh, Scotland*

GPR50 **is an orphan G protein-coupled receptor (GPCR) located on Xq28, a region previously implicated in multiple genetic studies of bipolar affective disorder (BPAD). Allele frequencies of three polymorphisms in *GPR50* were compared in case–control studies between subjects with BPAD (264), major depressive disorder (MDD) (226), or schizophrenia (SCZ) (263) and ethnically matched controls (562). Significant associations were found between an insertion/deletion polymorphism in exon 2 and both BPAD ($P = 0.0070$), and MDD ($P = 0.011$) with increased risk associated with the deletion variant (*GPR50*$^{\Delta 502-505}$). When the analysis was restricted to female subjects, the associations with BPAD and MDD increased in significance ($P = 0.00023$ and $P = 0.0064$, respectively). Two other single-nucleotide polymorphisms (SNPs) tested within this gene showed associations between: the female MDD group and an SNP in exon 2 ($P = 0.0096$); and female SCZ and an intronic SNP ($P = 0.0014$). No association was detected in males with either MDD, BPAD or SCZ. These results suggest that *GPR50*$^{\Delta 502-505}$, or a variant in tight linkage disequilibrium with this polymorphism, is a sex-specific risk factor for susceptibility to bipolar disorder, and that other variants in the gene may be sex-specific risk factors in the development of schizophrenia.**

Molecular Psychiatry (2005) **10,** 470–478. doi:10.1038/sj.mp.4001593
Published online 28 September 2004

Keywords: bipolar affective disorder; schizophrenia; G protein-coupled receptor; hypothalamus; pituitary; Xq28

Bipolar affective disorder (BPAD) is a severe psychiatric disorder affecting approximately 1% of the world's population, and shows no difference in lifetime prevalence between male and female subjects. Twin and adoption studies have demonstrated a strong genetic component, with a concordance in BPAD between monozygotic twins of 60%.[1] Major depressive disorder (MDD) has a lifetime prevalence of 17% with women twice as likely as men to develop the disorder.[2] Estimates of the heritability of MDD vary, but a meta-analysis of studies gives a point estimate of heritability of liability to MDD of 0.37.[3] Schizophrenia (SCZ), as with BPAD, has an estimated frequency of 1% in the population, but heritability estimates suggest that it has a larger genetic component than either BPAD or MDD, with monozygotic twins giving a point estimate of comorbidity of 0.81 in another recent meta-analysis.[4] Despite the strong genetic component in these major psychiatric disorders, there are also strong environmental influences.

Linkage to Xq28 has been studied many times in BPAD. Two loci, colour-blindness (CB), and glucose-6-phosphate dehydrogenase (G6PD), have been detected through linkage and association in more than one population.[5] Most significant are LOD scores of 8.1 and 7.35 between CB and BPAD in the American and Belgian populations, respectively, although reanalysis of much of the data from positive linkage results on the X chromosome has resulted in much reduced evidence for linkage and suggestions of ascertainment bias.[6–9] Several more recent studies have again renewed interest in the distal end of Xq, although the region implicated in these studies (Xq24–28) is larger than that depicted in the earlier reports.[5,10–13]

GPR50 is an X-linked orphan G protein-coupled receptor (also known as H9, melatonin-related receptor or ML1X) located on Xq28, and was initially cloned from a human pituitary cDNA library.[14,15] The gene encodes a protein of 617 amino acids that is 45% identical overall to human melatonin receptors

*Correspondence: Dr P Thomson, Medical Genetics Section, Department of Medical Sciences, University of Edinburgh, Molecular Medicine Centre, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU, Scotland.
E-mail: Pippa.Thomson@ed.ac.uk
Received 29 April 2004; revised 25 August 2004; accepted 5 August 2004

MTNR1A and MTNR1B, with identity increasing to 55% when comparing just the transmembrane domains.[15] Despite its close structural relationship to the melatonin receptors, GPR50 does not bind either [^{125}I]melatonin or [^{3}H]melatonin.[15,16]

RNA *in situ* hybridisation experiments with human GPR50 detected expression in the mediobasal hypothalamus, in a region containing the ventromedial and arcuate nuclei; and the paraventricular nucleus, as well as in the infundibular stalk.[15] Expression was also detected in the pituitary gland.[15] The hypothalamus has a punctate pattern of expression. In contrast, the expression pattern in the pituitary is widespread and heterogeneous, occurring in both the pars tuberalis and the pars distalis. The hypothalamus has been implicated in a number of physiological and behavioural responses. These include endocrine regulation (dorsal medial-ventromedial nuclei), reproduction, circadian and stress responses (dorsomedial hypothalamus), fluid balance, ingestive behaviours (arcuate nucleus and dorsomedial hypothalamus) and thermoregulation (lateral hypothalamus). The pituitary controls the endocrine system through secretion of hormones. This suggests a role for GPR50 and its ligand in neuroendocrine function.

The combination of map position on Xq28 and expression pattern makes GPR50 a positional and functional candidate in affective disorder.

Materials and methods

The study was approved by the Scottish Multicentre Research Ethics Committee.

Clinical assessment of patients

Subjects were in-patients or outpatients of hospitals in South East or South Central Scotland, and gave their informed consent for their inclusion in the study. Subjects were interviewed by an experienced psychiatrist and a blood was sample given for DNA extraction. Diagnoses were made according to DSM-IV criteria[17] based on case note review and interview using The Schedule for Affective Disorders and Schizophrenia — lifetime version (SADS-L).[18] Final diagnoses were reached by consensus between two trained psychiatrists (DB and WM). Age at onset was defined as the age when the subject first received professional help for psychiatric symptoms and 'early' onset was defined as less than 25 years of age.

Subjects in the group bipolar affective disorder (BPAD) included those with diagnoses Bipolar 1 and Bipolar 2, and all had a history of at least two episodes of major depression. Subjects included in the MDD group had a history of at least two episodes of major depression. The interview of subjects with MDD included additional questions to elicit symptoms consistent with 'bipolar spectrum disorder' as defined by Ghaemi *et al*.[19] The criteria for 'bipolar spectrum disorder' include: brief episodes of hypomanic symptoms of any duration less than 4 days; a first or second degree relative with bipolar disorder; symptoms of hypomania induced by antidepressant medication. In the first stages of analysis, subjects with MDD and 'bipolar spectrum' features were included in the group of major depression. The nosological status of 'bipolar spectrum' remains uncertain, but there are strong arguments for including these subjects in the bipolar group.[20] Analyses were therefore carried out with MDD excluding 'spectrum', and bipolar disorder including 'spectrum'.

Control subjects were drawn from the same population in South East and South Central Scotland, and recruited from donors for the Scottish National Blood Transfusion service or from hospital staff.

The composition of the samples available for the association study is given in Table 1.

Parents–offspring trios were ascertained by contacting parents through the probands. They gave informed consent for genetic studies and a venous blood sample was collected.

Genomic DNA was extracted from venous blood samples using standard protocols.

DNA sequencing

DNA sequencing was performed using ABI prism BigDye Terminator Cycle Sequencing Ready Reaction Kit. Purified PCR products were sequenced directly from 14 control individuals.

Genotyping

The insertion/deletion polymorphism (Δ502–505) was amplified using the following PCR: primers: 5′ TTCATTTCAAGCCTGCTTCC 3′, 5′ Hex.CTTAGGGTGGCTGGTAGTGG 3′. The fluorescently labelled PCR products were separated on an automated laser fluorescence DNA sequencer ABI 377 (Perkin-Elmer, Welleslay, MA, USA), and analysed using the GENESCAN (version 2.1) fragment-analysis software. The alleles were identified using the GENOTYPER program (version 2.0) (Perkin-Elmer).

The nonsynonymous single-nucleotide polymorphism (SNP), Val^{606}Ile (rs13440581), was genotyped using SNaPshot™ reactions carried out according to the manufacturer's instructions (Applied Biosystems). The PCR products were separated on an ABI PRISM® 377 DNA Sequencer and analysed using GENESCAN (version 2.1) fragment-analysis software. Genotypes of a subset of samples were confirmed using an ABI PRISM® 7900HT Sequence Detection

Table 1 Composition of available sample split into controls (CTL), bipolar (BPAD), major depressive disorder (MDD), schizophrenia (SCZ); bipolar spectrum (BPspec, a subdivision of MDD); male and female subjects

Samples	*CTL*	*BPAD*	*MDD*	*SCZ*	*BPspec*
Male	302	121	90	187	7
Female	260	143	136	76	15
Total	562	264	226	263	22

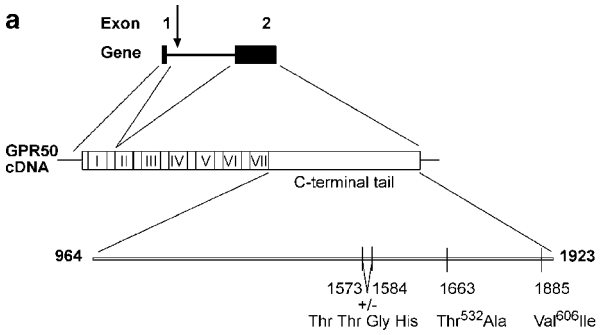
System and TaqMan technology. The SNP rs2072621 was available from ABI as an Assays-on-Demand™ TaqMan SNP genotyping assay. Genotypes were detected on an ABI PRISM® 7900HT Sequence Detection System.

For LD analysis, SNPs were either genotyped using the TaqMan ABI Assays-on-Demand™ system or by DNA sequencing performed using ABI prism BigDye Terminator Cycle Sequencing Ready Reaction Kit.

TaqMan genotyping was performed at the Wellcome Trust Clinical Research Facility, Genetics Core (http://www.wtcrf.ed.ac.uk/genetics/index.htm).

Statistical analysis

Observed genotype frequencies in female subjects for all case and control groups were tested for Hardy–Weinberg equilibrium using χ^2 tests with 1 df. Allele frequencies from both male and female chromosomes of their own patient group were used to calculate expected genotype frequencies. The χ^2 contingency table tests were used to test for allelic association with case–control status and for differences in allele frequencies between cases with maternal or paternal family history. Unconditional logistic regression[21] as implemented in UNPHASED[22] was used to test for association with two- and three-marker haplotypes (haplotype frequencies were estimated using the EM algorithm). UNPHASED was also used to calculate $|D'|$ and r^2 measures of linkage disequilibrium from trio (parents and offspring) genotypes.



b

Variant Name	Nucleotide change
Δ502-505	Deletion of 1573 → 1584 inclusive (ACC ACT GGC CAC)
$Thr^{532}Ala$	1663 A → G
$Val^{606}Ile$	1885 G → A

Figure 1 *GPR50* gene structure and position of polymorphisms. The *GPR50* gene structure is shown and polymorphisms identified by sequencing genomic DNA from 14 control individuals are illustrated (a). The seven transmembrane domains are denoted by roman numerals I-VII. An arrow denotes the approximate position of rs2072621. The nucleotide changes resulting in alterations of the amino-acid sequence for each polymorphism are shown in (b). Numbering of nucleotides is relative to the Ensembl transcript sequence ENST00000218316 and is with reference to the $GPR50^{wt}$ allele that encodes a full-length protein of 617 amino acids (a, b). The numbering of protein residues is relative to the 617 amino-acid $GPR50^{wt}$ as described by Ensembl protein ENSP00000218316.

Results

Sequencing and association study

Putative functional polymorphisms in *GPR50* were identified by sequencing the exons in 14 control individuals. No polymorphisms were detected in the first exon, but three nonsynonymous polymorphisms were detected in exon 2 that encodes transmembrane domains 2–7 and the carboxyl-terminal cytoplasmic tail (see Figure 1a). An in-frame 12 bp insertion/deletion polymorphism was identified beginning at nucleotide 1573, with respect to the Ensembl transcript sequence ENST00000218316, which results in the loss of four amino acids (Thr.Thr.Gly.His) and is designated Δ502–505. The 'insertion' allele, which contains Thr.Thr.Gly.His at positions 502–505, was found to be the most frequent form in the control population analysed and therefore this allele was designated $GPR50^{wt}$. $GPR50^{wt}$ encodes a full-length protein of 617 amino acids as annotated in the Ensembl database, ENSP00000218316. The Δ502–505 variant encodes a truncated protein of 613 amino acids as annotated in the Genbank and NCBI databases (gi:4758467, NM004224). In addition, two nonsynonymous polymorphisms were identified; a $Thr^{532}Ala$ (rs561077) and a $Val^{606}Ile$ (rs13440581). The nucleotide changes resulting in these amino-acid substitutions are detailed in Figure 1b. Analysis of genomic sequences indicated that variants Δ502–505 and $Thr^{532}Ala$ are in complete linkage disequilibrum with each other, but not with $Val^{606}Ile$. Association analysis was therefore performed on the polymorphisms Δ502–505 and $Val^{606}Ile$, as well as an intronic SNP described below.

Association between the $GPR50^{wt/\Delta 502-505}$ polymorphism and psychiatric illness was explored in a case–control association study in a population sampled from the South East of Scotland (Table 1). None of the female genotype frequencies in the control or case groups differed significantly from Hardy–Weinberg equilibrium. Significant association was seen between Δ502–505 polymorphism and both MDD and BPAD (see Table 2; $P=0.011$ and $P=0.0070$). No association was seen between Δ502–505 and SCZ ($P=0.12$). The female association was stronger than that for the combined group when the cases were subdivided by gender (MDD, $P=0.0064$ and BPAD, $P=0.00023$). No association was seen for either *GPR50* variant and any male case group.

The difference in allele frequency between male and female controls (43.4 *vs* 37.6% Table 2) is not significant $P=0.11$ and, if the allele frequency in BPAD female subjects is compared to the combined allele frequency of male and female controls, the association remains significant ($P=0.00089$). Genotype relative risks are 3.1 (95% confidence interval 1.7–5.6) for the $GPR50^{\Delta 502-505}$ homozygote and 1.5 (95% confidence interval 0.9–2.5) for the heterozygote compared to the $GPR50^{wt}$ homozygote. This

Table 2 Single marker association

		All			*Male*			*Female*			*Female MDD not BPspec*			Female BPspec		
Marker	*Samples*	*P-value*	*Frequency*	*N*[a]	*P-value*	*Frequency*	*N*[a]	*P-value*	*Frequency*	*N*[a]	*P*-value	Frequency	*N*[a]	*P-value*	*Frequency*	*N*[a]
Δ502–505	CTL		39.7	786		43.4	288		37.6	498						
	BPAD	**0.0070**	47.9	401	0.53	40.0	115	***0.00023***	51.0	286						
	MDD	**0.011**	47.7	352	0.48	47.7	86	**0.0064**	47.7	266	**0.044**	45.3	236	***0.0015***	66.7	30
	SCZ	0.12	44.7	331	0.15	50.3	181	0.92	38.0	150						
	BPAD + BPspec							***0.000026***	52.5	316						
Val[606]Ile	CTL		38.5	793		42.3	293		36.2	500						
	BPAD	0.24	42.0	400	0.74	40.5	116	0.077	42.6	284						
	MDD	**0.040**	44.9	354	0.99	42.2	90	**0.0096**	45.8	264	**0.018**	45.3	236	0.14	50.0	28
	SCZ	0.30	41.8	335	0.84	43.2	185	0.40	40.0	150						
	BPAD + BPspec							**0.044**	43.3	312						
rs2072621	CTL		43.7	767		44.9	285		42.9	482						
	BPAD	0.61	42.1	387	0.79	43.5	115	0.71	41.5	272						
	MDD	0.58	45.5	341	0.19	53.1	81	0.97	43.1	260	0.57	45.2	230	0.080	26.7	30
	SCZ	0.31	47.3	273	0.16	37.9	153	***0.0014***	59.2	120						
	BPAD + BPspec							0.42	40.1	302						

[a]*N* = number of chromosomes.
P-values <0.05 in bold, *P*-values <0.0056 in bold italics.

implies additive allelic action on the scale of risk, which is consistent with the lack of deviation from Hardy–Weinberg equilibrium in BPAD female subjects. The frequencies of the $GPR50^{\Delta 502-505}$ homozygote, heterozygote and $GPR50^{wt}$ homozygote in BPAD female subjects *vs* controls are 27.3 *vs* 13.7%, 47.8 *vs* 47.6% and 25.2 *vs* 38.6%, implying a low penetrance value for the $GPR50^{\Delta 502-505}$ homozygote of 2.0% (penetrance $= 0.273 \times 0.01/0.137$), assuming a population frequency for BPAD of 1%.

Linkage disequilibrium map of GPR50

In order to define the haplotype blocks involved in this association, and to exclude any neighbouring genes, further markers were genotyped on a panel of 31 BPAD parent–offspring trios with affected probands. The linkage disequilibrium map of *GPR50* was constructed using 10 SNPs spanning 21.5 kb (Table 3). Despite *GPR50* being only 5 kb in length, two independently segregating haplotype blocks were observed. The location of the break between these blocks was resolved to 286 bp between markers rs12558086 and rs12557475 in the single intron ($|D'| = 0.04$). The haplotype block containing the Δ502–505 polymorphism spans 4.5 kb covering part of the intron, all of exon 2 and approximately 2 kb of the 3′ region. This suggests that the haplotype block carrying the putative functional polymorphism in BPAD is limited to *GPR50*, although we cannot formally rule out the possibility that the association results from another gene in linkage disequilibrium with these markers.

Additional marker association results

In order to investigate the association further, we genotyped two additional polymorphisms: $Val^{606}Ile$ (rs13440581), identified by sequencing 14 control samples (see Figure 1) and rs2072621 identified from the public databases[23] (Table 2). For both of these polymorphisms, the genotype frequencies in the female groups did not differ significantly from those expected under Hardy–Weinberg equilibrium. Weak association was detected between the exon 2 $Val^{606}Ile$ polymorphism and the female MDD sample ($P = 0.0096$), this SNP showed no significant associations with female BPAD or SCZ. The intronic SNP rs2072621 gave no association with either BPAD or MDD, but showed significant association in the female SCZ sample ($P = 0.0014$). As for the Δ502–505 polymorphism, no significant associations were found between $Val^{606}Ile$ or rs2072621 and disease status in males. Investigation of two- and three-marker haplotypes gave significant association with disease status in female MDD, BPAD and SCZ, although none of these associations were significantly stronger than their initial single marker association (Table 4). No association was seen for any male case group between disease status and haplotype.

Statistical considerations

If the *P*-value for declaration of significance ($P = 0.05$) is adjusted for multiple testing, the Bonferroni corrected *P*-value for declaration of significance accounting for the nine tests in the initial study (three sex categories by three diagnosis categories on a single marker) is $P = 0.0056$. Under this conservative cutoff, association is declared for female BPAD and the Δ502–505 polymorphism, and for female schizophrenics and the intronic SNP rs2072621. Power analysis of the male sample suggests that the lack of association within males is unlikely to be due to the smaller number of chromosomes tested. The sample of 115 BPAD and 288 control male chromosomes for which the insertion/deletion genotypes were obtained has 74 or 100% power to detect an association, assuming a haplotype risk of 1.5 or 3.1, respectively, allele frequency of 0.4 and type I error of 5%. This suggests that the lack of association found in males is

Table 3 Pairwise measures of $|D'|$ and r^2 calculated from 31 trios with BPAD probands using both transmitted and untransmitted chromosomes for 10 markers spanning the region 19.2 and 2.2 kb up- and downstream from $GPR50^{\Delta 502-505}$

	Marker	*Inter-marker distance bp*	*% Min Allele*		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
									[D'][a]					
1	rs180496	1509	32.8			**1.00**	0.68	0.11	0.26	0.07	0.21	0.00	0.05	0.51
2	rs501938	7277	24.1		0.16		0.58	0.33	0.35	0.62	0.04	0.39	0.03	0.33
3	rs529286	6762	31.5		0.11	0.22		**0.71**	0.26	0.23	0.13	0.03	0.09	0.24
4	rs2072621	1382	46.6		0.01	0.04	0.28		0.53	0.08	0.08	0.06	0.05	0.31
5	rs12558086	286	14.3	r^2	0.01	0.07	0.02	0.06		0.04	**1.00**	0.32	0.40	0.08
6	rs12557475	87	26.0		0.00	0.03	0.01	0.00	0.00		**0.71**	0.53	0.51	0.20
7	rs1201872	1945	22.0		0.03	0.00	0.00	0.00	0.05	0.39		0.40	0.40	**1.00**
8	Δ502–505	301	42.3		0.00	0.07	0.00	0.00	0.01	0.07	0.04		0.56	0.31
9	$Val^{606}Ile$	1912	42.0		0.00	0.00	0.01	0.00	0.02	0.06	0.04	0.28		0.27
10	rs512294	—	10.7		0.02	0.04	0.01	0.01	0.00	0.00	0.04	0.02	0.01	

[a][D'] value >0.70 in bold.

Table 4 Two- and three-marker haplotype associations (*P*-values)

Marker	*Samples*	*All*	*Male*	*Female*	*Female MDD not BPspec*	*Female BPspec*
rs2072621/Δ502–505	BPAD	**0.017**	0.87	***0.0018***		
	MDD	0.055	0.36	**0.035**	0.16	***0.0016***
	SCZ	0.16	0.30	**0.014**		
	BPAD + BPspec			***0.00023***		
Δ502–505/Val^{606}Ile	BPAD	**0.039**	0.91	***0.0011***		
	MDD	0.10	0.67	**0.027**	0.095	**0.019**
	SCZ	0.18	0.30	**0.014**		
	BPAD + BPspec			***0.00023***		
rs2072621/Val^{606}Ile	BPAD	0.51	0.99	**0.19**		
	MDD	0.069	0.27	**0.046**	0.071	**0.0065**
	SCZ	0.63	0.76	**0.010**		
	BPAD + BPspec			**0.099**		
3 marker	BPAD	0.13	0.94	**0.0093**		
	MDD	0.14	0.60	**0.091**	0.21	***0.0051***
	SCZ	0.078	0.068	**0.014**		
	BPAD + BPspec			***0.0026***		

P-values < 0.05 in bold, *P*-values < 0.0056 bold italics.

Table 5 Analysis of female GPR50$^{\Delta 502-505}$ association

Diagnosis	*Samples*	*N*[a]	*Deletion frequency*	*P-value*
Control	All	498	37.6	
BPAD	All	286	51.0	***0.00023***
	Early onset	150	52.0	***0.00016***
	Maternal	74	60.8	***0.00014***
	Paternal	64	59.4	***0.00080***
MDD	All	266	47.7	**0.0064**
	Early onset	56	46.4	0.20
	Maternal	52	40.4	0.69
	Paternal	20	55.0	0.12

P-values < 0.05 in bold, *P*-values < 0.0056 bold italics.
[a]N = number of chromosomes.

unlikely to be due to the smaller number of X-chromosomes tested in males (115 chromosomes in male, 286 chromosomes in female subjects).

Parent of origin/age of onset analysis

Evidence for a female only association led us to investigate parent of origin effects (see Donald *et al*[24]). The female BPAD group was divided into those reporting major psychiatric disorders in one or more first- or second-degree relatives on their maternal ($N = 37$) or paternal ($N = 32$) families (Table 5). Allele frequencies between these groups did not differ ($P = 0.86$). This suggests that there is no parent of origin effect in the association between BPAD and the Δ502–505 polymorphism. Age at onset was examined for the female BPAD identifying those with an early age of onset (24 years or less) of affective illness ($N = 75$ BPAD). Association between early age of onset BPAD and Δ502–505 polymorphism was significant (Table 5, $P = 0.00016$). Parent of origin and early age of onset groups were also defined for MDD. No significant associations were found with any of these groups (Table 5).

The number of observations in each class in the analyses of parent of origin and age of onset was quite small, these results should, therefore, be regarded as preliminary and interpreted with caution.

'Bipolar spectrum' analysis

Analysis revealed that the MDD association was largely driven by a group ($N = 15$) fulfilling diagnostic criteria for bipolar spectrum disorder (BPspec, $P = 0.0015$), and that the remaining MDD sample ($N = 232$) gave only a weakly significant association ($P = 0.044$; Table 2). When female bipolar spectrum disorder and BPAD cases are combined, the association between affected status and the Δ502–505 polymorphism is further increased ($P = 0.000026$, Tables 2 and 4).

Discussion

This is the first study investigating a possible link between putative functional polymorphisms in GPR50, an orphan G protein-coupled receptor, and major mental illness. Both the putative functional polymorphisms studied result in alterations of the amino-acid sequence in the carboxyl-terminus cytoplasmic tail. The deletion allele, Δ502–505, which is associated with both BPAD and MDD (global *P*-values 0.007 and 0.011), results in the deletion of four

amino-acid residues, including two threonine residues (see Figure 1). The SNP at nucleotide 1885 is characterised by a single base-pair transition (G→A), resulting in a nonsynonymous Val[606]Ile amino-acid substitution (rs13440581). A third SNP at position 1663, which is in complete LD with Δ502–505, also results in a nonsynonymous Thr[532]Ala amino-acid substitution (rs561077) in the carboxyl tail. GPR50 has an unusually long carboxyl tail of >300 amino acids, longer than that for any other known GPCR. The carboxyl tail contains 41 serine and threonine residues and may be involved in receptor coupling to G proteins, tethering the receptor to the plasma membrane, receptor sequestration, desensitisation and downregulation.[25,26] The identification of a consensus site for a serine protease in the carboxyl tail also raises the possibility that it has enzymatic function, which may depend on receptor activation/phosphorylation state.[15] Increasing numbers of proteins in addition to the G proteins are now known to interact with GPCRs.[27] Many of these GPCR-associated proteins interact with the third intracellular loop or with the C-terminal cytoplasmic tail. A mutation in the distal portion of the β2-adrenergic receptor has been shown to disrupt a PDZ domain-mediated interaction, resulting in reduced receptor recycling and increased targeting to lysosomes.[28] Cao *et al*[28] also showed that amino-acid substitution of the serine residue Ser411, a potential site of phosphorylation by GRKs in the intact receptor, effects endocytic sorting to recycling or lysosomal pathways. Recently, Barrett *et al*[29] have reported an association between a polymorphism in the G protein receptor kinase 3 gene (GRK3) and bipolar disorder. GRKs phosphorylate serine and threonine residues in the cytoplasmic tail or third intracellular loop of GPCRs in the presence of high agonist concentrations. Phosphorylation results in rapid termination of signalling through homologous desensitisation.[30] Two of the polymorphisms identified, Δ502–505 and Thr[532]Ala, disrupt threonine residues and may therefore disrupt sites of GRK phosphorylation. The third coding polymorphism, Val[606]Ile, alters no known protein motifs, but may well alter downstream signalling or protein–protein interactions in the cytoplasmic tail through as yet uncharacterised interactions. There is no clear functional effect of the intronic SNP rs2072621; however, in other genes, intronic SNPs have been shown to alter splicing efficiency (eg Liu *et al*[31]).

The clear association with female subjects affected with major mental illness and not with male subjects is intriguing. There is no reported gender bias in the numbers of individuals affected by BPAD. MDD however is twice as common in female as compared to male subjects. A number of other groups have reported sex-specific results with X-linked candidate genes in both BPAD[5,13,32–35] and MDD.[36] A study of affected sib pairs by Stine *et al*.[37] reported excess allele sharing in affected sister–sister pairs in the Xq26–28 region that was not found in affected brother–brother or brother–sister pairs. This suggests that despite the lack of a sex bias in the numbers of individuals affected by BPAD, the genetic risk factors may differ between genders.

Analysis of the female genotype relative risk in our study suggests an additive effect of each allele on risk. Non-pseudoautosomal Xq28 genes are reported to undergo X-inactivation[38] and therefore it is surprising to observe this effect. The additive effect of the *GPR50* deletion in female subjects could in theory result from the differences between numbers of cells expressing the deletion form in heterozygotes, when the X-chromosomes are inactivated randomly, compared to expression of the deletion form in all cells in Δ502–505 homozygotes. Until the X-inactivation status of *GPR50* is assessed directly, this remains speculative. This hypothesis would not explain the lack of association with BPAD in males carrying a single copy of the Δ502–505 *GPR50* gene. However, another gene in this region, BGN, is reported to be expressed in a sex chromosome dosage-dependant manner despite being X-inactivated and having no copy on the Y-chromosome.[39] Geerkens *et al*[39] suggest that BGN protein expression may be controlled during transcription or translation by another sex chromosome-linked gene that escapes X-inactivation. In a similar manner, this mechanism could account for the additive effect of the deletion in female subjects observed in this study. Alternatively, studies of X–Y gene pairs suggest that some X-linked genes may be expressed at higher levels in the brains of female compared to male subjects, independently of X-inactivation status, at least in mice.[40] It is important, therefore, that the expression levels of GPR50 in male and female subjects are studied at both RNA and protein levels before effects of protein levels on phenotype are discounted.

Other possibilities for the observed sex-specific pattern of association include imprinting and interactions with hormones. Imprinting on the X-chromosome has been shown to influence phenotype in the study of Turner's syndrome patients who inherit an XO karyotype.[41] Skuse *et al*[41] compared the levels of social cognition of women with Turner's syndrome who had inherited the maternal X-chromosome, compared to those who had inherited the paternal X-chromosome. Patients inheriting the maternal X-chromosome tended to show more social difficulties, including offensive and disruptive behaviour. Although genetic and epigenetic mechanisms are possible, these remain speculative. A perhaps more obvious possibility is a female-specific hormonal interaction, given the expression of GPR50 in both the hypothalamus and the pituitary.

The endogenous ligand and physiological role for GPR50 still remain to be identified. The localised expression of this receptor in hypothalamus and pituitary could, however, suggest a role for GPR50 in neuroendocrine function and HPA (hypothalamic-pituitary-adrenocortical) axis regulation. There are multiple lines of evidence which suggest that abnor-

mally elevated HPA axis activity and raised cortisol levels are associated with the development of mood disorders, including MDD and BPAD.[42–44] There is also considerable interplay between the HPA and the hypothalamo-pituitary-gonadal axes and certain reproductive hormones, including oestrogen and progesterone, have been implicated in the regulation of mood and the pathogenesis of psychiatric disease disorders (reviewed in Toren *et al*[45], Halbreich *et al*[46]). The mechanisms behind these effects are not fully understood but one could speculate that interactions between gonadal hormones and the hypothalamus and pituitary could provide a possible mechanism to explain the female-only association for *GPR50*. Interestingly, another female-specific association has recently been reported in the literature and this identified a variant of the CREB gene which displayed significant evidence for linkage to familial recurrent early-onset major depression in female but not in male subjects.[47,48] Zubenko *et al*[48] reported an LOD score of 8.19 in a region containing the CREB1 gene, which encodes a cAMP responsive element-binding protein (CREB). The CREB protein is thought to interact with oestrogen receptors to promote the transcription of genes containing oestrogen responsive elements, even in the absence of CREB-binding sites. This mechanism could implicate CREB with sex-specific patterns of gene expression.[49]

We have shown that *GPR50* is a strong candidate gene for female-associated affective disorder, and that there is weaker evidence of an association with schizophrenia also in female subjects. In conclusion, our findings support the hypothesis of linkage between bipolar-related affective disorders and Xq28.

Acknowledgements

We thank Peter Visscher for statistical advice and discussion, and Kirsty Millar for critical reading of this manuscript. This work was supported by Organon Laboratories Ltd, Newhouse, Lanarkshire, UK.

References

1 Kendler KS, Pedersen N, Johnson L, Neale MC, Mathe AA. A pilot Swedish twin study of affective illness, including hospital- and population-ascertained subsamples. *Arch Gen Psychiatry* 1993; **50**: 699–700.
2 Jones I, Kent L, Craddock N. The genetics of affective disorders. In: McGuffin P, Owen MJ, Gottesman II (eds) *Psychiatric Genetics and Genomics*. Oxford university press: Oxford, England, 2002.
3 Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry* 2000; **157**: 1552–1562.
4 Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. *Arch Gen Psychiatry* 2003; **60**: 1187–1192.
5 Massat I, Souery D, Del-Favero J, Oruc L, Noethen MM, Blackwood D *et al.* Excess of allele1 for alpha3 subunit GABA receptor gene (GABRA3) in bipolar patients: a multicentric association study. *Mol Psychiatry* 2002; **7**: 201–207.
6 Hebebrand J, Hennighausen K. A critical analysis of data presented in eight studies favouring X-linkage of bipolar illness with special emphasis on formal genetic aspects. *Hum Genet* 1992; **90**: 289–293.
7 Baron M, Freimer NF, Risch N, Lerer B, Alexander JR, Straub RE *et al.* Diminished support for linkage between manic depressive illness and X-chromosome markers in three Israeli pedigrees. *Nat Genet* 1993; **3**: 49–55.
8 Berrettini W. Progress and pitfalls: bipolar molecular linkage studies. *J Affect Disord* 1998; **50**: 287–297.
9 Baron M. Genetic linkage and bipolar disorder: a cautionary note. *J Affect Disord* 2001; **67**: 267–273.
10 Bocchetta A, Piccardi MP, Martinelli V, Quesada G, Del Zompo M. Maternal inheritance of manic depression in hemizygotes for the G6PD-Mediterranean mutation. Indirect evidence for Xq28 transmission in Sardinia. *Psychiatr Genet* 1999; **9**: 63–68.
11 Pekkarinen P, Terwilliger J, Bredbacka PE, Lonnqvist J, Peltonen L. Evidence of a predisposing locus to bipolar disorder on Xq24–q27.1 in an extended Finnish pedigree. *Genome Res* 1995; **5**: 105–115.
12 Ekholm JM, Kieseppa T, Hiekkalinna T, Partonen T, Paunio T, Perola M *et al.* Evidence of susceptibility loci on 4q32 and 16p12 for bipolar disorder. *Hum Mol Genet* 2003; **12**: 1907–1915.
13 Muller DJ, Schulze TG, Jahnes E, Cichon S, Krauss H, Kesper K *et al.* Association between a polymorphism in the pseudoautosomal X-linked gene SYBL1 and bipolar affective disorder. *Am J Med Genet* 2002; **114**: 74–78.
14 Gubitz AK, Reppert SM. Assignment of the melatonin-related receptor to human chromosome X (GPR50) and mouse chromosome X (Gpr50). *Genomics* 1999; **55**: 248–251.
15 Reppert SM, Weaver DR, Ebisawa T, Mahle CD, Kolakowski Jr LF. Cloning of a melatonin-related receptor from human pituitary. *FEBS Lett* 1996; **386**: 219–224.
16 Conway S, Drew JE, Mowat ES, Barrett P, Delagrange P, Morgan PJ. Chimeric melatonin mt1 and melatonin-related receptors. Identification of domains and residues participating in ligand binding and receptor activation of the melatonin mt1 receptor. *J Biol Chem* 2000; **275**: 20602–20609.
17 American Psychiatric Association. 1994. *Diagnostic and Statistical Manual of Mental Disorders*, (4th Edn). APA: Washington, DC.
18 Endicott J, Spitzer RL. A Diagnostic interview: the schedule for affective disorders and schizophrenia. *Arch Gen Psych* 1978; **35**: 837–862.
19 Ghaemi SN, Ko JY, Goodwin FK. 'Cade's disease' and beyond: misdiagnosis, antidepressant use, and a proposed definition for bipolar spectrum disorder. *Can J Psychiatry* 2002; **47**: 125–134.
20 Smith DJ, Harrison N, Muir W, Blackwood DHR. Bipolar spectrum disorders in young adults with recurrent depression: comparison of structured diagnostic criteria with a manic symptoms checklist. *J Affect Disord* 2004 (in press).
21 Zhao JH, Curtis D, Sham PC. Model-free analysis and permutation tests for allelic associations. *Hum Hered* 2000; **50**: 133–139.
22 Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* 2003; **25**: 115–221.
23 UCSC Genome Browser.http://genome.ucsc.edu/.
24 Donald JA, Salmon JA, Adams LJ, Littlejohn T, Maher A, Mitchell PB *et al.* Parental sex effects in bipolar affective disorder pedigrees. *Genet Epidemiol* 1997; **14**: 611–616.
25 Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol Rev* 2001; **53**: 1–24, Review.
26 Chuang TT, Iacovelli L, Sallese M, De Blasi A. G protein-coupled receptors: heterologous regulation of homologous desensitization and its implications. *Trend Pharmacol Sci* 1996; **17**: 416–421.
27 Brady AE, Limbird LE. G protein-coupled receptor interacting proteins: emerging roles in localization and signal transduction. *Cell Signal* 2002; **14**: 297–309.
28 Cao TT, Deacon HW, Reczek D, Bretscher A, von Zastrow M. A kinase-regulated PDZ-domain interaction controls endocytic sorting of the beta2-adrenergic receptor. *Nature* 1999; **401**: 286–290.
29 Barrett TB, Hauger RL, Kennedy JL, Sadovnick AD, Remick RA, Keck PE *et al.* Evidence that a single nucleotide polymorphism in the promoter of the G protein receptor kinase 3 gene is associated with bipolar disorder. *Mol Psychiatry* 2003; **8**: 546–557.

30 Bunemann M, Hosey MM. G-protein coupled receptor kinases as modulators of G-protein signalling. *J Physiol* 1999; **517**(Part 1): 5–23.
31 Liu HX, Cartegni L, Zhang MQ, Krainer AR. A mechanism for exon skipping caused by nonsense or missense mutations in BRCA1 and other genes. *Nat Genet* 2001; **27**: 55–58.
32 Rubinsztein DC, Leggo J, Goodburn S, Walsh C, Jain S, Paykel ES. Genetic association between monoamine oxidase A microsatellite and RFLP alleles and bipolar affective disorder: analysis and meta-analysis. *Hum Mol Genet* 1996; **5**: 779–782.
33 Ho LW, Furlong RA, Rubinsztein JS, Walsh C, Paykel ES, Rubinsztein DC. Genetic associations with clinical characteristics in bipolar affective disorder and recurrent unipolar depressive disorder. *Am J Med Genet* 2000; **96**: 36–42.
34 Saito T, Parsia S, Papolos DF, Lachman HM. Analysis of the pseudoautosomal X-linked gene SYBL1in bipolar affective disorder: description of a new candidate allele for psychiatric disorders. *Am J Med Genet* 2000; **96**: 317–323.
35 Hawi Z, Mynett-Johnson L, Gill M, Murphy V, Straubl RE, Kendler KS *et al*. Pseudoautosomal gene: possible association with bipolar males but not with schizophrenia. *Psychiatr Genet* 1999; **9**: 129–134, Erratum in: *Psychiatr Genet* 1999; **9**: 209.
36 Schulze TG, Muller DJ, Krauss H, Scherk H, Ohlraun S, Syagailo YV *et al*. Association between a functional polymorphism in the monoamine oxidase A gene promoter and major depressive disorder. *Am J Med Genet* 2000; **96**: 801–803.
37 Stine OC, McMahon FJ, Chen L, Xu J, Meyers DA, MacKinnon DF *et al*. Initial genome screen for bipolar disorder in the NIMH genetics initiative pedigrees: chromosomes 2, 11, 13, 14, and X. *Am J Med Genet* 1997; **74**: 263–269.
38 Carrel L, Cottle AA, Goglin KC, Willard HF. A first-generation X-inactivation profile of the human X chromosome. *Proc Natl Acad Sci USA* 1999; **96**: 14440–14444.
39 Geerkens C, Vetter U, Just W, Fedarko NS, Fisher LW, Young MF *et al*. The X-chromosomal human biglycan gene BGN is subject to X inactivation but is transcribed like an X–Y homologous gene. *Hum Genet* 1995; **96**: 44–52.
40 Xu J, Burgoyne PS, Arnold AP. Sex differences in sex chromosome gene expression in mouse brain. *Hum Mol Genet* 2002; **11**: 1409–1419.
41 Skuse DH, James RS, Bishop DV, Coppin B, Dalton P, Aamodt-Leeper G *et al*. Evidence from Turner's syndrome of an imprinted X-linked locus affecting cognitive function. *Nature* 1997; **387**: 705–708.
42 Stokes PE. The potential role of excessive cortisol induced by HPA hyperfunction in the pathogenesis of depression. *Eur Neuropsychopharmacol* 1995; **5**: 77–82.
43 Rybakowski JK, Twardowska K. The dexamethasone/corticotropin-releasing hormone test in depression in bipolar and unipolar affective illness. *J Psychiatr Res* 1999; **33**: 363–370.
44 Pariante CM. Depression, stress and the adrenal axis. *J Neuroendocrinol* 2003; **15**: 811–812.
45 Toren P, Dor J, Rehavi M, Weizman A. Hypothalamic-pituitary-ovarian axis and mood. *Biol Psychiatry* 1996; **40**: 1051–1055.
46 Halbreich U, Lumley LA. The multiple interactional biological processes that might lead to depression and gender differences in its appearance. *J Affect Disord* 1993; **29**: 159–173.
47 Zubenko GS, Hughes III HB, Maher BS, Stiffler JS, Zubenko WN, Marazita ML. Genetic linkage of region containing the CREB1 gene to depressive disorders in women from families with recurrent, early-onset, major depression. *Am J Med Genet* 2002; **114**: 980–987.
48 Zubenko GS, Hughes III HB, Stiffler JS, Brechbiel A, Zubenko WN, Maher BS *et al*. Sequence variations in CREB1 cosegregate with depressive disorders in women. *Mol Psychiatry* 2003; **8**: 611–618.
49 Lazennec G, Thomas JA, Katzenellenbogen BS. Involvement of cyclic AMP response element binding protein (CREB) and estrogen receptor phosphorylation in the synergistic activation of the estrogen receptor by estradiol and protein kinase activators. *J Steroid Biochem Mol Biol* 2001; **77**: 193–203.