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ARTICLE

Polymorphisms in wolframin (WFS1) gene are possibly related to increased risk for mood disorders

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Abstract

Wolfram syndrome gene (WFS1) has been suggested to have a role in the susceptibility for mood disorders. A 26-fold increased risk for psychiatric disorders in WFS1 mutation carriers has been suggested. In this study we tested the hypothesis that the WFS1 gene is related to the risk for mood disorders. We analysed 28 single-nucleotide polymorphisms (SNPs) of the WFS1 gene in 224 unrelated patients with major depressive disorder and bipolar disorder and in 160 healthy control subjects. Patients were further stratified according to their comorbidity with anxiety disorders. We applied arrayed primer extension (APEX)-based genotyping technology followed by association and haplotype analysis. Five SNPs in the WFS1 gene were associated with major depressive disorder, and three SNPs with bipolar disorder. Haplotype analysis revealed a common GTA haplotype, formed by SNPs 684C/G, 1185C/T and 1832G/A, conferring risk for affective disorders. Specifically, for major depression the GTA haplotype has an OR of 1.59 ($p=0.01$) and for bipolar disorder an OR of 1.89 ($p=0.03$). These results support the hypothesis that the WFS1 gene is involved in the genetic predisposition for mood disorders.

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Key words: Association, bipolar disorder, genetics, haplotype analysis, major depressive disorder, single-nucleotide polymorphism (SNP), WFS1, wolframin.

Introduction

Wolfram syndrome (MIM 222300) is a rare autosomal recessive neurodegenerative disorder, characterized by diabetes insipidus, diabetes mellitus, optic atrophy and deafness (acronym DIDMOAD). The characteristic symptoms include juvenile-onset diabetes mellitus and progressive bilateral optic atrophy (Kinsley et al., 1995). Patients may later develop diabetes insipidus and deafness, as well as a range of neurological and psychiatric abnormalities, including dementia, psychosis and affective disorder (Kinsley et al., 1995; Swift et al., 1990). Allele variants of Wolfram syndrome gene (WFS1) have been suggested to play a role in susceptibility to hearing impairment (Cryns et al., 2002), diabetes mellitus (Awata et al., 2000) and psychiatric disorders (Swift et al., 1991).

The gene for Wolfram syndrome, WFS1, has been identified in chromosomal region 4p16 (Inoue et al., 1998; Strom et al., 1998). Genetic analysis has demonstrated that mutations in the WFS1 gene are clearly associated with the DIDMOAD syndrome (Hardy et al., 1999). The WFS1 gene consists of eight exons encompassing 33.4 kb of genomic DNA encoding a polypeptide (wolframin) of 890 amino acids with an apparent molecular mass of 100 kDa. Wolframin is a tetrameric protein possessing nine predicted transmembrane segments (Hofmann et al., 2003). Northern

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Table 1. Demographic and clinical characteristics of subjects in current study

Characteristic	MDD	MDA	MD	BPD	BPA
n	177	48	69	47	35
Sex (M/F)	39/138	14/34	16/53	21/26	12/23
Age (yr), mean ± S.D.	40.3 ± 13.5	41.2 ± 12.2	40.3 ± 15.0	35.4 ± 12.7	35.5 ± 11.9
Range (yr)	18–73	18–63	18–73	17–65	17–61

Psychiatric subjects were divided by diagnostic categories as follows: MDD, major depressive disorder extended; all cases with major depressive disorder, includes pure phenotype as well phenotypes with comorbid anxiety disorder [panic disorder, generalized anxiety disorder (GAD), obsessive–compulsive disorder (OCD), social phobia]. MDA, major depressive disorder with comorbid anxiety disorder (GAD, OCD, social phobia) except panic disorder. MD, major depressive disorder without any comorbid disorder. BPD, bipolar disorder extended; includes 12 patients with bipolar disorder without any comorbid disorder and 35 bipolar disorder patients with comorbid anxiety disorder (panic disorder, GAD, OCD, social phobia). BPA, bipolar disorder with comorbid anxiety disorder (panic disorder, GAD, OCD, social phobia).

blot analysis has revealed prominent expression of wolframin mRNA in affected tissues, including the brain and pancreas (Inoue et al., 1998). These expression sites correlate with the atrophic changes associated with the syndrome. Recent study suggests the importance of wolframin in the regulation of intracellular Ca^{2+} homeostasis (Osman et al., 2003). However, the precise function of this protein remains to be established.

As the WFS1 gene resides in chromosomal region 4p16, it is a challenging target for psychiatric research. Linkage studies implicate this region as harbouring the putative susceptibility gene for bipolar disorder (Blackwood et al., 1996). Heterozygous carriers of the gene for Wolfram syndrome are predisposed to psychiatric disorders as shown by the above-average psychiatric hospitalization among the blood relatives of Wolfram syndrome patients (Swift et al., 1990). This hypothesis was further confirmed by genetic analysis of the WFS1 locus in families with Wolfram syndrome where a 26-fold increased risk was established (Swift et al., 1998). Further studies have not uniformly confirmed the association between variations in the WFS1 gene and mood disorders (Crawford et al., 2002; Evans et al., 2000). On the other hand, a single-nucleotide polymorphism (SNP) at position 1832 has been shown to be associated with suicide and impulsive behaviour (Sequeira et al., 2003). In the present study we attempted to clarify the role of the WFS1 gene in predicting risk for mood disorders. We analysed 28 SNPs of the WFS1 gene in patients with major depressive disorder (MDD) and in patients with bipolar disorder (BPD). Association and haplotype analysis were performed in order to establish the effect of genetic variations in the WFS1 gene on the risk for mood disorders.

Methods

Subjects and psychiatric assessment

Unrelated patients ($n = 224$) with MDD and with BPD were recruited in the study along with healthy control individuals ($n = 160$) from the Estonian population. Diagnoses of patients were substantiated by psychiatric interview and verified by Mini International Neuropsychiatric Interview (MINI 5.0.0) based on DSM-IV (Sheehan et al., 1998). There were no cases with Wolfram syndrome and no known family history of Wolfram syndrome among the study subjects. Controls were evaluated using MINI to exclude those with psychiatric morbidity, and with a family history interview to exclude those with a known history of major psychiatric disorders in first-degree relatives. There were no significant differences in demographics between patients and healthy volunteers in terms of age and sex. Clinical demographic characteristics are presented in Table 1.

MDD and BPD diagnoses were considered separately in subsequent analyses under the hypothesis that genetic variability of the WFS1 gene may contribute to these two disorders in different ways. MDD and BPD groups were subdivided on the assumption that a range of psychiatric manifestations – paranoid delusions, severe depression, attempted suicides, poor impulse control, chronic anxiety and/or panic attacks have been described in Wolfram syndrome homozygotes and heterozygotes (Swift et al., 1998). Because of the high rate of anxiety comorbidity in the patient population, the comparisons were subsequently done with both the entire sample of patients (MDD or BPD) and between the stratified diagnostic categories.

Psychiatric subjects were divided by diagnostic categories as follows (see Table 1): MDD – major

depressive disorder extended; all cases with major depressive disorder, includes pure phenotype as well as phenotypes with comorbid anxiety disorder [panic disorder, generalized anxiety disorder (GAD), obsessive–compulsive disorder (OCD), social phobia] ($n=177$); MDA – major depressive disorder with comorbid anxiety disorder (GAD, OCD, social phobia) except panic disorder ($n=48$); MD – major depressive disorder without any comorbid disorder ($n=69$); BPD – bipolar disorder extended, includes 12 patients with bipolar disorder without any comorbid disorder and 35 bipolar disorder patients with comorbid anxiety disorder (panic disorder, GAD, OCD, social phobia) ($n=47$); BPA – bipolar disorder with comorbid anxiety disorder (panic disorder, GAD, OCD, social phobia) ($n=35$).

Patients were recruited among consecutive outpatients and in-patients at the Clinic of Psychiatry of Tartu University Clinics, and controls by newspaper advertisement in Tartu, Estonia. The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Review Committee on Human Research of the University of Tartu. Each subject provided written informed consent.

Genotyping and sample preparation

SNP detection was performed by arrayed primer extension (APEX) technology. APEX is a genotyping and resequencing technology that combines the advantages of dideoxy sequencing with the parallelization and high-throughput potential of microarray format (Kurg et al., 2000). APEX technology is suitable for SNP detection allowing the analysis of hundreds of SNPs in one sample and SNP profiling. Detailed information about the studied polymorphisms is presented in Table 2.

Standard high-salt extraction method was used to isolate genomic DNA from 9 ml venous blood samples. Amplification of WFS1 genomic fragments was performed in eight individual PCR reactions using touchdown conditions (primers for each PCR reaction are listed in Table 3). A 20% fraction of the dTTP in the amplification mixture was substituted by dUTP, allowing later fragmentation of PCR products with uracil-*N*-glycosylase.

Pooled amplification products were concentrated and purified, followed by fragmentation and functional inactivation of the unincorporated dNTPs as described in Kurg et al. (2000). Production of oligonucleotide microchips and APEX reactions were performed as described earlier (Kurg et al., 2000). Polymorphisms were identified by Genorama™ 4.1 genotyping software (Asper Biotech Ltd, Tartu, Estonia) by using signal patterns from wild-type DNA sequences as the reference.

Statistical analysis

Association analysis was performed using GENEPOP Version 3.3 software (Raymond and Rousset, 1995). Allele frequencies were compared by association tests separately for MDD and BPD phenotypes: control subjects vs. subjects with broad phenotype (MDD and BPD) and subsequently smaller subgroups. p values for allelic and genotypic association were calculated using Fisher's exact test. The significance level for all statistical tests was 0.05. Haplotype analysis was performed using the maximum-likelihood method for simultaneously estimating haplotype frequencies and haplotype–phenotype association as described by Tregouet et al. (2002). Pairwise linkage disequilibrium (LD) was estimated by a log-linear model and the extent of disequilibrium was expressed in terms of the standardized D' characteristic. The maximum power for the MDD sample reached 65% and for the BPD sample 70% ($p=0.05$) according to the actual sample size and observed risk allele frequencies.

Results

We genotyped 28 polymorphisms (26 SNPs and 2 deletions) in WFS1 gene in 224 unrelated patients and 160 healthy controls. Given the relatively small number of subjects with BPD and MDD subphenotypes, overlapping analyses served to maximize the likelihood of finding differences by diagnostic subcategory, if any existed, in studied population. On the other hand, such stratification helps to define subtype-specific SNPs or SNPs reflecting the general risk for mood disorders.

Association analysis

In our screening set, five markers displayed a nominal association with broadly defined major depression phenotypes ($p<0.05$) and three markers with BPD phenotypes ($p<0.05$). We compared allele frequencies in the control and affective patients groups stratified under MDD and BPD phenotypes. The genotype frequencies for each polymorphism in affected and control groups did not deviate significantly from the Hardy–Weinberg equilibrium. The statistical data for informative SNPs are presented in Tables 4–6.

Table 2. Description of single nucleotide polymorphisms (SNPs) in the WFS1 gene used in our study

Gene and SNP	Position from ATG	Other names	Exon	db SNP rs #	Allele 1	Allele 2	AA	AA comment	Allele 1 frequency	Allele 2 frequency
WFS1 406	WFS1 11622		4	rs # na	C	T	Q136X	Nonsynon ch	0.99	0.01
WFS1 460	WFS1 11676		4	rs # na	G	A		5′ splice signal	0.97	0.03
WFS1 505	WFS1 13786		5	rs # na	G	A	E169K	Nonsynon ch	0.89	0.11
WFS1 676	WFS1 14506		6	rs # na	C	T	Q226X	Nonsynon ch	0.99	0.01
WFS1 684	WFS1 14514		6	rs7672995	C	G	R228R	Synon ch	0.54	0.46
WFS1 874	WFS1 23214		8	rs # na	C	T	P292S	Nonsynon ch	0.99	0.01
WFS1 887	WFS1 23227		8	rs # na	T	G	I296S	Nonsynon ch	0.98	0.02
WFS1 935	WFS1 23275	WFS1 937	8	rs # na	T	G	M312R	Nonsynon ch	0.80	0.20
WFS1 997	WFS1 23337		8	rs1801212	A	G	I333V	Nonsynon ch	0.68	0.32
WFS1 1023	WFS1 23363		8	rs # na	C	T	F341F	Synon ch	0.90	0.10
WFS1 1185	WFS1 23525		8	rs1801206	C	T	V395V	Synon ch	0.48	0.52
WFS1 1287	WFS1 23627		8	rs # na	C	T	C429C	Synon ch	0.99	0.01
WFS1 1294	WFS1 23634	WFS1 1296	8	rs # na	C	G	L432V	Nonsynon ch	0.95	0.05
WFS1 1321	WFS1 23661	WFS1 1323	8	rs # na	G	A	V441M	Nonsynon ch	0.89	0.11
WFS1 1367	WFS1 23707		8	rs1801208	G	A	R456H	Nonsynon ch	0.94	0.06
WFS1 1549	WFS1 23889		8	rs # na	del	C		del517fs/ter521	0.99	0.01
WFS1 1645	WFS1 23985		8	rs # na	C	T	L549L	Synon ch	0.96	0.04
WFS1 1832	WFS1 24172		8	rs734312	G	A	R611H	Nonsynon ch	0.53	0.47
WFS1 2206	WFS1 24546		8	rs # na	G	A	G736S	Nonsynon ch	0.91	0.09
WFS1 2254	WFS1 24594		8	rs # na	G	T	E752X	Nonsynon ch	0.99	0.01
WFS1 2314	WFS1 24654		8	rs # na	C	T	R772C	Nonsynon ch	0.98	0.02
WFS1 2322	WFS1 24662		8	rs2230721	G	A	K774K	Synon ch	0.93	0.07
WFS1 2433	WFS1 24773		8	rs1046314	A	G	K811K	Synon ch	0.56	0.44
WFS1 2565	WFS1 24905		8	rs1046316	G	A	S855S	Synon ch	0.63	0.37
WFS1 2596	WFS1 24936	WFS1 2598	8	rs3821945	G	A	D866N	Nonsynon ch	0.99	0.01
WFS1 2611	WFS1 24951	WFS1 2613	8	rs # na	G	A	V871M	Nonsynon ch	0.93	0.07
WFS1 2642	WFS1 24982		8	rs # na	del	TC		del882fs/ter937	0.95	0.05
WFS1 2763	WFS1 25103		3′-UTR	rs # na	G	A	nc	3′-UTR	0.92	0.08

rs # na – SNP is not listed in NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/); db SNP rs #, accession number of SNP in NCBI dbSNP database; AA, amino acid; Nonsynon ch, non-synonymous change; Synon ch, synonymous change; nc, non-coding.

Table 3. Primers used to amplify genomic regions for APEX analysis

Primer name	Forward primer 5′→3′	Reverse primer 5′→3′	Product size (bp)
WFS 4	TCGGAGAATCTGGAGGCTGA	CATTACAAGCTGCTCAACCC	253
WFS 5	ACAAGGCCTTTGACCACATC	GTGCCCAGGGTGAATCCTC	225
WFS 6	CTATGATCCCCAGAACGTAGGA	CAGAACACTGAGCCCCAAAC	419
WFS 8A	CCTCGTTCCCACGTACCATC	GTAGCAGTAGGTGCCCTTGA	766
WFS 8B	CCTGGTCGTCCTCAATGTCA	CATAGAACCAGCAGAACAGC	447
WFS 8CD	TGGTTCACGTCTCTGGAGCT	GAACTTCTTGATGTGGCAGG	549
WFS 8E	CTGGATGCGCTGCCTCTACG	TCAGGCCGCCGACAGGAATG	350
WFS 8H	GAGTTCAGCACCATCCTGGAG	ACAGCAGCCTTCCCTTTGTCG	381

Major depressive disorder (*MDD*)

Patients with MDD were stratified according to co-morbidity to define possible subtype-specific SNPs in the WFS1 gene for MDD. Two SNPs in the WFS1 gene, 1185C/T and 2206G/A, were associated with the presence of major depression without any comorbid disorders (MD group). In the MDA group (MD with

Table 4. Results of association analysis of WFS1 polymorphisms in major depressive disorder

	Allele			Allelic P			Allele 2 frequencies			
SNP	1	2	Exon	MD	MDA	MDD	MD	MDA	MDD	Controls
684	C	G	6	0.08	0.09	0.007	0.50	0.52	0.52	0.41
935	T	G	8	0.62	0.01	0.19	0.20	0.11	0.18	0.22
1023	C	T	8	0.11	0.02	0.02	0.07	0.04	0.07	0.12
1185	C	T	8	0.04	0.15	0.01	0.58	0.56	0.58	0.47
1645	C	T	8	1	0.05	0.55	0.04	0	0.03	0.04
1832	G	A	8	0.15	0.25	0.17	0.52	0.51	0.50	0.45
2206	G	A	8	0.02	0.38	0.04	0.04	0.08	0.06	0.10
2565	G	A	8	0.08	0.63	0.04	0.32	0.38	0.33	0.41

SNP, Single-nucleotide polymorphism. MD, Major depressive disorder without any comorbid disorder. MDA, major depressive disorder with comorbid anxiety disorder [generalized anxiety disorder (GAD), obsessive–compulsive disorder (OCD), social phobia] except panic disorder. MDD, major depressive disorder extended; all cases with major depressive disorder, includes pure phenotype as well phenotypes with comorbid anxiety disorder (panic disorder, GAD, OCD, social phobia).

Table 5. Results of association analysis of WFS1 polymorphisms in bipolar disorder

	Allele			Allelic P		Allele 2 frequencies		
SNP	1	2	Exon	BPA	BPD	BPA	BPD	Controls
684	C	G	6	0.02	0.005	0.58	0.59	0.41
1023	C	T	8	0.05	0.12	0.04	0.06	0.12
1185	C	T	8	0.12	0.05	0.58	0.59	0.47
1832	G	A	8	0.68	0.09	0.48	0.55	0.45
2565	G	A	8	0.40	0.05	0.35	0.29	0.41

SNP, Single-nucleotide polymorphism. BPA, Bipolar disorder with comorbid anxiety disorder [panic disorder, generalized anxiety disorder (GAD), obsessive–compulsive disorder (OCD), social phobia]. BPD, bipolar disorder extended; includes 12 patients with bipolar disorder without any comorbid disorder and 35 bipolar disorder patients with comorbid anxiety disorder (panic disorder, GAD, OCD, social phobia).

comorbid anxiety disorder but without panic disorder) we found associations with SNPs 935T/G, 1023C/T and 1645C/T. In the broadest group (all cases with MDD with all comorbid anxiety disorders) we detected associations with SNPs 684C/G, 1023C/T, 1185C/T, 2206G/A and 2565G/A (Table 4). Comparison of genotype frequencies detected significantly more SNP 684G/G ($p = 0.008$) and SNP 1185T/T genotypes ($p = 0.008$) among MDD patients compared to controls (Table 6).

Bipolar disorder (*BPD*)

Patients with BPD were stratified into two subgroups – BPA (cases of BPD with comorbid anxiety disorders) and BPD (includes group BPA and also patients with only BPD). In the BPA group two SNPs (684C/G and 1023C/T) were associated with clinical phenotypes. Positive associations with broad bipolar phenotypes were established with SNPs 684C/G, 1185C/T and 2565G/A (Table 5). Analysis of genotype

Table 6. Genotype frequencies of single-nucleotide polymorphisms (SNPs) selected for haplotype analysis

SNP	Genotypes		
684C/G	C/C	C/G	G/G
Controls	0.39	0.40	0.21
MDD ($p=0.008$)	0.26	0.46	0.28
BPD ($p=0.005$)	0.17	0.49	0.34
1185C/T	C/C	C/T	T/T
Controls	0.32	0.43	0.25
MDD ($p=0.008$)	0.20	0.44	0.36
BPD ($p=0.06$)	0.21	0.41	0.38
1832G/A	G/G	G/A	A/A
Controls	0.31	0.49	0.20
MDD ($p=0.17$)	0.25	0.52	0.23
BPD ($p=0.09$)	0.13	0.66	0.21

p values estimating genotype differences between cases and controls are given.
MDD, major depressive disorder extended; all cases with major depressive disorder, includes pure phenotype as well phenotypes with comorbid anxiety disorder (panic disorder, GAD, OCD, social phobia).
BPD, Bipolar disorder extended; includes 12 patients with bipolar disorder without any comorbid disorder and 35 bipolar disorder patients with comorbid anxiety disorder (panic disorder, GAD, OCD, social phobia).

frequencies detected significantly more of 684G/G genotype among BPD patients compared to controls ($p=0.005$) (Table 6).

Haplotype analysis

Haplotype analysis was performed according to a pairwise LD pattern in broadly defined major depression (MDD) (cases + controls, $n=337$) and BPD (cases + controls, $n=207$) datasets. Presence of pairwise LD ($D'>0.6$) in both affected and control groups was used as a criterion to include SNP markers for haplotype analysis. The overall SNP call rate in a study sample reached 99%. Several SNP markers showing association with MDD or BPD were not included in the haplotype analysis due to their low allelic frequency in the affected group (<10%) or poor LD with flanking polymorphisms. All statistically relevant data about detected haplotype–phenotype associations with MDD and BPD are presented in Tables 7 and 8. Figure 1 gives an additional illustration of the SNPs analysed in our study and the haplotype structure.

Major depressive disorder (*MDD*)

In the case of the MDD phenotype, eight haplotypes were found based on the three most polymorphic SNPs (684C/G, 1185C/T, 1832G/A) (Table 7, Figure 1). Six haplotypes were present with probabilities higher than 2%, the global p value for haplotypic association with MDD was 0.027 ($\chi^2=12.63$, d.f. = 5). The reference haplotype (HT1) combined the major alleles at each locus, while another major haplotype (HT2) combined the minor alleles. Taken together with haplotype HT3, these three common haplotypes constituted more than 80% of the alleles in MDD patients and controls. HT1 (CCG) was more frequent in control subjects (44.3%) compared to cases (33.7%), whereas HT2 (GTA) was over-represented in the affected group. Haplotype 3 (CTA) was the only one that was almost equally represented both in cases and controls. Haplotypes HT4–HT6 were enriched in affected individuals, expressing haplotype effect associated with increased risk of depression (OR $\geqslant$ 2). Haplotypes HT7 and HT8 were rare.

Haplotype 2 (GTA) was significantly associated with a higher risk of MDD (OR 1.59, $p=0.01$) compared to the reference haplotype (CCG). Other haplotypes (HT4–HT6) showed only tentative associations with MDD. With HT4 (GCG) the higher relative risk was found for individuals carrying the 684G allele (OR 2.02, $p=0.06$) compared to the reference haplotype (CCG), while with HT5 (CTG) a higher relative risk for individuals carrying the 1185T allele (OR 2.01, $p=0.07$) compared to the reference haplotype was established.

Bipolar disorder (*BPD*)

In the case of BPD eight haplotypes were found based on the three most polymorphic SNPs (684C/G, 1185C/T, 1832G/A) (Table 8, Figure 1). Seven haplotypes were present with probabilities higher than 2%, the global p value for haplotypic association with BPD was 0.034 ($\chi^2=15.17$, d.f. = 7). The reference haplotype (HT1) combined the major alleles at each locus, while another major haplotype (HT2) combined the minor alleles. Together with haplotype HT3 these three haplotypes constituted more than 80% of all alleles in controls, but only 75% of all alleles in cases. HT1 was over-represented in control subjects (44.3%) compared to cases (28.9%), whereas HT2 was more frequent in the affected group. Unlike the MDD group, HT3 (CTA) and HT4 (GCG) were over-represented in controls similarly to the reference haplotype. Haplotypes HT5–HT7 were more frequent in affected individuals, expressing haplotype effect associated with increased

Table 7. Results of haplotype analysis in patients with major depressive disorder

	Single-nucleotide polymorphism			Haplotype frequency			
HT	684C/G	1185C/T	1832G/A	Controls	Patients	Haplotypic OR (95% CI)	*p*
1	C	C	G	44.3	33.7	*	
2	G	T	A	31.0	38.9	1.587 (1.116–2.255)	0.010
3	C	T	A	9.3	8.3	1.216 (0.666–2.223)	0.530
4	G	C	G	5.1	7.4	2.024 (0.970–4.223)	0.060
5	C	T	G	3.6	5.9	2.015 (0.937–4.386)	0.072
6	G	T	G	2.7	4.1	2.107 (0.935–4.811)	0.075
7	G	C	A	2.1	0.7	–	–
8	C	C	A	1.9	1.0	–	–

Table 8. Results of haplotype analysis in patients with bipolar disorder

	Single-nucleotide polymorphism			Haplotype frequency			
HT	684C/G	1185C/T	1832G/A	Controls	Patients	Haplotypic OR (95% CI)	*p*
1	C	C	G	44.3	28.9	*	
2	G	T	A	31.0	39.5	1.890 (1.074–3.325)	0.027
3	C	T	A	9.3	5.5	0.732 (0.214–2.531)	0.626
4	G	C	G	5.1	3.3	0.834 (0.196–3.537)	0.803
5	C	T	G	3.6	5.8	2.477 (0.892–7.139)	0.092
6	G	T	G	2.7	7.7	3.797 (1.123–12.43)	0.033
7	G	C	A	2.1	8.0	4.251 (1.225–14.75)	0.023
8	C	C	A	1.9	1.3	1.076 (0.112–10.38)	0.950

risk of BPD (OR $\geqslant$ 2). HT6 and HT7 were clearly more frequent in BPD patients compared to the MDD study group.

Haplotype 2 (GTA) was associated with a higher risk of BPD (OR 1.89, $p = 0.03$) by comparison to the reference haplotype (CCG). Unlike with MDD, HT4 (GCG) did not show any association with relative risk of BPD. With HT5 (CTG) a tentative evidence of a higher relative risk for individuals carrying the 1185T allele (OR 2.48, $p = 0.09$) compared to the reference haplotype (CCG) was established. Interestingly, HT6 (GTG) and HT7 (GCA) were quite common in cases, both clearly indicating associations with a higher risk of BPD: HT6 (OR 3.80, $p = 0.03$) and HT7 (OR 4.25, $p = 0.02$).

Discussion

In the present study we analysed 28 SNPs in the WFS1 gene in patients with MDD and BPD compared to healthy control subjects. We found significant associations between affective disorder phenotypes and several SNPs and defined the WFS1 haplotype(s) related to an increased risk for psychiatric disorders. The most prominent effect was established with SNP at position 684C/G. It is a synonymous variation and does not change the composition of the wolframin peptide (R228R). This variation was significantly associated only with the broad phenotype (MDD), and could, therefore, possibly be related to the general risk for mood disorders. Indeed, this SNP was also associated with an increased risk for BPD. Differences in 684 genotype distributions gave additional support to this finding. MDD and BPD patients had significantly more 684G/G genotype compared to controls ($p = 0.008$ and $p = 0.005$ respectively, Table 6). Another SNP specifically associated with the MDD subphenotype, but also with bipolar disorder broad phenotype (BPD) was 2565G/A (S855S). Interestingly, both these SNPs have also been described in the Wolfram syndrome family and in patients with type 2 diabetes, but its functional relevance is not known

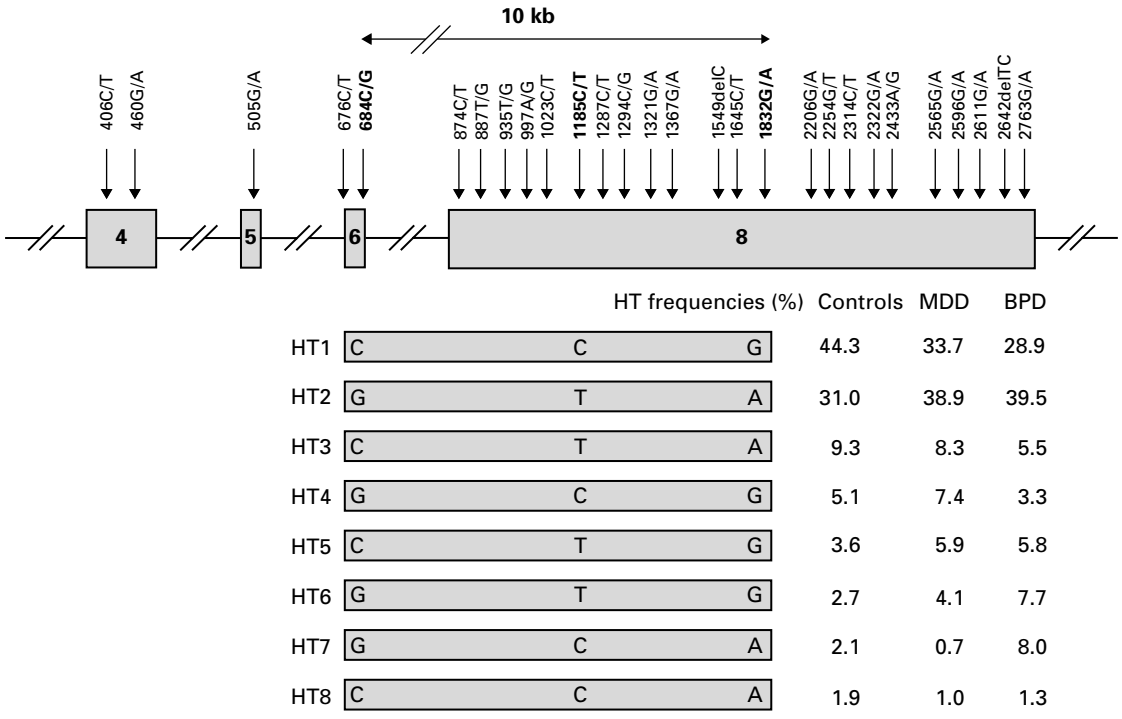

Figure 1. Single nucleotide polymorphisms and haplotypes in the WFS1 gene and their frequencies in different groups.

(Hardy et al., 1999; Minton et al., 2002). Another SNP associated significantly with the MDD phenotype was synonymous variant 1185C/T (V395V). This SNP was also associated with an increased risk for MD and BPD. The 1185 genotype distribution was significantly different in case of MDD compared to controls and this outcome gives further support to the relevance of association. Significant associations with SNP 2206G/A were found in the MDD broad phenotype and in the MD phenotype similarly to 1185C/T. A missense SNP at position 935T/G (M312R) was associated only with the presence of MDD with anxiety disorder, likewise SNP 1645C/T (L549L), while 935T/G has been described earlier in patients with schizophrenia (Torres et al., 2001). To our knowledge 1645C/T has not been studied before. Synonymous variation at position 1023C/T (F341F) showed associations with MDD phenotypes comorbid with anxiety disorders (MDA and MDD) as well as with BPD phenotypes comorbid with anxiety disorders. Possibly this marker may be connected more to anxiety disorders than mood disorders. No associations with suicide were found previously (Crawford et al., 2002).

After Bonferroni correction, none of described marker-disease associations remained statistically significant. We emphasize that the limited size of our patient (especially in case of BPD) population provides insufficient power to detect weak effects, therefore, replication studies with larger and independent samples are needed. We cannot exclude a hypothesis that the described polymorphisms are in LD with other functionally significant polymorphisms, which could actually be involved in mood disorders.

Haplotype analysis confirmed the presence of a risk haplotype for MDD and BPD in the WFS1 gene. Eight haplotype combinations were found with three SNPs (684C/G, 1185C/T and 1832G/A, with a genomic distance of 10 kb) in linkage disequilibrium (Figure 1). The GTA haplotype was associated with a higher risk for MDD (OR 1.59) and for bipolar affective disorder (OR 1.89). This finding is in accordance with the hypothesis that variations in the WFS1 gene are related to psychiatric disorders (Sequeira et al., 2003; Swift et al., 1998). The R611 allele (1832G) was found to be associated with suicidal and impulsive behaviour (Sequeira et al., 2003). This result was further confirmed by finding that bipolar patients with the R611/R611 genotype had a significantly higher mean number of suicide attempts than those with other genotypes in this position (Cryns et al., 2003; Li et al., 2002).

In our study we found a slight over-representation of the 1832A (H611) allele in bipolar patients compared to controls due to the higher frequency of heterozygous 1832G/A genotype, and much lower frequency of 1832G/G genotype (0.66 vs. 0.49 and 0.13 vs. 0.31 respectively), whereas in a previous study a higher frequency of 1832G (R611) variant has been found (Sequeira et al., 2003). On the other hand, a similar allelic distribution to our study (H611 or 1832A more frequent in patients with depression) has been described (Furlong et al., 1999; Kato et al., 2003; Ohtsuki et al., 2000). Different allelic distributions could be explained by different populations and by the high heterozygosity of this SNP. In our population, frequencies of 1832G and 1832A alleles were 0.55 and 0.45 respectively.

In several previous studies, no associations between SNPs in the WFS1 gene and mood disorders have been found (Evans et al., 2000; Middle et al., 2000; Ohtsuki et al., 2000), therefore, it is probably not a major susceptibility gene for psychiatric disorders. However, it remains possible that WFS1 variants substantially raise the susceptibility to mood disorders. Haplotype analysis of our study revealed significant associations and an increased risk for MDD and BPD associated with GTA haplotype. Importantly, this haplotype has risk effects for both subtypes of mood disorders. Haplotype analysis improves the power of association studies, thus, earlier negative findings could be explained by differences in the design of analysis (previous studies have been mostly single SNP association studies) (Middle et al., 2000). On the other hand, even if the WFS1 gene is not directly related to susceptibility, our haplotype analysis provides additional support to the importance of the 4p16 chromosomal region in the development of psychiatric disorders. This region has in several studies been shown to be involved in genetic predisposition for psychiatric disorders (Blackwood et al., 1996). In addition, positive associations have been found between psychiatric disorders (mainly with schizophrenia) and SNPs in cholecystokinin 1 receptor and dopamine 5 receptor genes (Muir et al., 2001; Wei and Hemmings, 1999). These genes are located very closely (distances of 20 and 3.2 Mb respectively) in the same chromosomal region, 4p16, and give further supportive evidence for the importance of this region in the susceptibility to psychiatric disorders. However, more detailed studies to confirm our present findings are necessary.

In conclusion, our study supports the role of the WFS1 gene in susceptibility for MDD and BPD. By means of haplotype analysis we were able to define the GTA haplotype in the WFS1 gene related to an increased risk for mood disorders.

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Statement of Interest

None.

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