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ARTICLE

Analysis of SNP profiles in patients with major depressive disorder

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Abstract

The present study focused on 91 single-nucleotide polymorphisms (SNPs) in 21 candidate genes to find associations with major depressive disorder (MDD). In total, 160 healthy controls and 177 patients with MDD were studied. We applied arrayed primer extension (APEX) based genotyping technology followed by association and haplotype analysis. SNPs in CCKAR, DRD1, DRD2, and HTR2C genes showed nominally significant associations with MDD. None of these associations remained significant after adjustment for multiple testing. Haplotype analysis revealed CCKAR haplotypes to be associated with MDD (global $p=0.004$). More precisely, we found the GAGT haplotype to be associated with increased risk for MDD (OR 7.42, 95% CI 2.13–25.85, $p=0.002$). This haplotype effect remained significant after Bonferroni correction ($p=0.04$ after Bonferroni's adjustment). Altogether we were able to find some nominal associations, but due to small sample size these results should be taken as exploratory. However, the effect of GAGT haplotype on the CCKAR gene may be considered as increasing the risk for MDD.

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

Introduction

Mood disorders are among the most prominent causes of disability and the second leading source of disease burden (Merikangas et al., 2002; Murray and Lopez, 1996). Most epidemiological and family studies indicate that the lifetime prevalence of unipolar major depressive disorder (MDD) is between 5% and 10% (Moldin et al., 1991). Suicide has been reported to occur in 10–15% of patients previously hospitalized for depression, a rate of death that is three orders of magnitude greater than that reported for the American population as a whole (Angst et al., 1999; Zubenko et al., 2002). Therefore, MDD is obviously a serious problem for public health.

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Family and twin studies demonstrate that genetic factors typically account for 40–50% of the risk for developing MDD (McGuffin et al., 1996). A large number of family studies have demonstrated an increased risk of MDD among relatives of MDD probands, with ~2-fold increased risk in first-degree relatives (Kupfer et al., 1989; McGuffin et al., 1991). However, several reports do not support so high genetic risk for MDD, indicating the importance of environmental factors (Sullivan et al., 2000).

The aim of our study was to screen a set of single-nucleotide polymorphisms (SNPs) for their association with MDD. We defined the genes and their variations which have been previously published in the literature and yielded some (although inconsistent) significant findings, as candidate genes in our study. Genes related to the following neurotransmitter systems were included in the present survey: cholecystokinin (CCK), opioid peptides (OP), serotonin (5-HT) and dopamine (DA). CCK has been extensively studied as

Table 1. Description of single-nucleotide polymorphisms (SNPs) analysed in our study

Gene name (abbreviation)	Gene and SNP	Position from ATG	Location	db SNP rs #	Allele 1	Allele 2	Function	Allele 1 frequency
Cholecystokinin (CCK)	CCK −45	CCK −1172	3p22–p21.3	rs1799923	C	T	5′-UTR	0.89
	CCK 1270	CCK −9	3p22–p21.3	rs754635	C	G	5′-UTR	0.85
	CCK 6662	CCK 5386	3p22–p21.3	rs3774396	C	T	intron	0.98
Cholecystokinin A receptor (CCKAR)	CCKAR −128	CCKAR −333	4p15.1–p15.2	rs1800908	G	T	5′-UTR	0.96
	CCKAR 201	CCKAR −286	4p15.1–p15.2	rs1799723	A	G	5′-UTR	0.94
	CCKAR 246	CCKAR −241	4p15.1–p15.2	rs # n.a.	G	A	5′-UTR	0.97
	CCKAR 608	CCKAR 122	4p15.1–p15.2	rs1800856	G	A	intron	0.96
	CCKAR 1260	CCKAR 773	4p15.1–p15.2	rs1800855	T	A	intron	0.71
	CCKAR 1266	CCKAR 779	4p15.1–p15.2	rs1800857	T	C	intron	0.76
	CCKAR 3849	CCKAR 8231	4p15.1–p15.2	rs1805037	C	T	I296I	0.99
Cholecystokinin B receptor (CCKBR)	CCKBR −215	CCKBR −216	11p15.4	rs1799721	C	A	5′-UTR	0.95
	CCKBR 109	CCKBR 109	11p15.4	rs1805000	C	T	L37F	0.93
	CCKBR 1550	CCKBR 9962	11p15.4	rs1805002	G	A	V125I	0.92
	CCKBR 2491	CCKBR 10907	11p15.4	rs1800843 rs8192470	C	A	Intron	0.88
Dopamine receptor D1 (DRD1)	DRD1 −2218	DRD1 −2218	5q35.1	rs # n.a.	T	C	5′-UTR	0.94
	DRD1 −2102	DRD1 −2102	5q35.1	rs # n.a.	C	A	5′-UTR	0.93
	DRD1 −2030	DRD1 −2030	5q35.1	rs # n.a.	T	C	5′-UTR	0.97
	DRD1 −1251	DRD1 −1252	5q35.1	rs # n.a.	G	C	5′-UTR	0.86
	DRD1 −800	DRD1 −800	5q35.1	rs265981	T	C	5′-UTR	0.38
	DRD1 −94	DRD1 −94	5q35.1	rs5326	G	A	5′-UTR	0.84
	DRD1 −48	DRD1 −48	5q35.1	rs4532	G	A	5′-UTR	0.44
Dopamine receptor D2 (DRD2)	DRD2 −241	DRD2 −50978	11q23	rs1799978	A	G	5′-UTR	0.78
	DRD2 −141	DRD2 −50878	11q23	rs1799732	C	del	5′-UTR	0.84
	DRD2 −7054	DRD2 −7053	11q23	rs # n.a.	C	A	5′-UTR	0.92
	DRD2 −913	DRD2 −913	11q23	rs1079597	A	G	5′-UTR	0.32
	DRD2 −901	DRD2 −901	11q23	rs1079598	C	T	5′-UTR	0.32
	DRD2 286	DRD2 287	11q23	rs # n.a.	T	C	intron	0.93
	DRD2 3625	DRD2 3626	11q23	rs2734834	A	T	intron	0.49
	DRD2 3785	DRD2 3786	11q23	rs1800498	C	T	intron	0.39
	DRD2 11924	DRD2 11890	11q23	rs1801028	C	G	S311C	0.93
	DRD2 11997	DRD2 11915	11q23	rs6277	T	C	P319P	0.94
	DRD2 16893	DRD2 16891	11q23	rs2234689	C	G	3′-UTR	0.72
	DRD2 24470	DRD2 24546	11q23	rs1800497	C	T	K713E (in ANKK1 gene)	0.80
Dopamine receptor D3 (DRD3)	DRD3 −707	DRD3 −710	3q13.3	rs1800828	G	C	5′-UTR	0.71
	DRD3 −343	DRD3 −346	3q13.3	rs1800827	G	A	5′-UTR	0.96
	DRD3 25	DRD3 25	3q13.3	rs6280	A	G	G9S	0.69
Dopamine receptor D4 (DRD4)	DRD4 −1217	DRD4 −1216	11p15.5	rs # n.a.	G	del	5′-UTR	0.62
	DRD4 −809	DRD4 −808	11p15.5	rs936461	G	A	5′-UTR	0.80
	DRD4 −768	DRD4 −767	11p15.5	rs4987058	G	A	5′-UTR	0.86
	DRD4 −616	DRD4 −615	11p15.5	rs747302	C	G	5′-UTR	0.68
	DRD4 −521	DRD4 −521	11p15.5	rs1800955	C	T	5′-UTR	0.41
	DRD4 −376	DRD4 −376	11p15.5	rs916455	C	T	5′-UTR	0.96
Dopamine receptor D5 (DRD5)	DRD5 1481	DRD5 1481	4p16.1	rs1967551	C	T	3′-UTR	0.65
Tyrosine hydroxylase (TH)	TH 241−243	TH 2066	11p15.5	rs6356	G	A	V81M	0.61
	TH 614	TH 3891	11p15.5	rs # n.a.	T	C	L205P	0.96
5-hydroxytryptamine (serotonin) receptor 1A (HTR1A)	HTR1A −1018	HTR1A −1019	5q11.2–q13	rs6295	C	G	5′-UTR	0.43
	HTR1A −480	HTR1A −480	5q11.2–q13	rs # n.a.	A	del	5′-UTR	0.91
5-hydroxytryptamine	HTR1B	HTR1B −1089	6q13	rs1778258	T	C	5′-UTR	0.24

Table 1 (*cont.*)

Gene name (abbreviation)	Gene and SNP	Position from ATG	Location	db SNP rs #	Allele 1	Allele 2	Function	Allele 1 frequency
(serotonin) receptor 1B (HTR1B)	HTR1B	HTR1B −700	6q13	rs1228814	C	A	5′-UTR	0.55
	HTR1B −511	HTR1B −511	6q13	rs130056	G	T	5′-UTR	0.995
	HTR1B −161	HTR1B −161	6q13	rs130058	A	T	5′-UTR	0.78
	HTR1B 129	HTR1B 129	6q13	rs6298	C	T	S43S	0.74
	HTR1B 276	HTR1B 276	6q13	rs130059	G	A	A92A	0.96
	HTR1B 371	HTR1B 371	6q13	rs130060	T	G	F124C	0.99
	HTR1B 705	HTR1B 705	6q13	rs130062	C	T	A235A	0.80
	HTR1B 861	HTR1B 861	6q13	rs6296	G	C	V287V	0.74
	HTR1B	HTR1B 1180	6q13	rs6297	G	A	3′-UTR	0.23
5-hydroxytryptamine (serotonin) receptor 2A (HTR2A)	HTR2A −1438	HTR2A −1437	13q14–q21	rs6311	A	G	5′-UTR	0.42
	HTR2A 73	HTR2A 74	13q14–q21	rs1805055	C	A	T25N	0.98
	HTR2A 102	HTR2A 102	13q14–q21	rs6313	T	C	S34S	0.37
	HTR2A 1354	HTR2A 61008	13q14–q21	rs6314	C	T	H452Y	0.94
5-hydroxytryptamine (serotonin) receptor 2C (HTR2C)	HTR2C 68	HTR2C 4390	Xq24	rs6318	G	C	C23S	0.83
	HTR2C 2831	HTR2C 181359	Xq24	rs1801412	T	G	3′-UTR	n.a.
5-hydroxytryptamine (serotonin) receptor 3A (HTR3A)	HTR3A 1302	HTR3A −507	11q23.1–q23.2	rs1150226	T	C	5′-UTR	0.31
	HTR3A 1596	HT3A 14378	11q23.1–q23.2	rs1176713	G	A	L459L	0.26
Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (SLC6A4)	SLC6A4	SLC6A4 18784	17q11.1–q12	rs6352	A	C	K605N	0.96
	SLC6A4	SLC6A4 10647	17q11.1–q12	rs6353	G	A	T439T	0.92
	SLC6A4	SLC6A4 167	17q11.1–q12	rs6355	G	C	G56A	0.77
Tryptophan hydroxylase 1 (tryptophan 5-monooxygenase) (TPH1)	TPH1 218	TPH1 14494	11p15.3–p14	rs1800532	A	C	intron	0.29
	TPH1 779	TPH1 15055	11p15.3–p14	rs1799913	A	C	intron	0.27
Opioid receptor, mu 1 (OPRM1)	OPRM1 31	OPRM1 50665	6q24–q25	rs # n.a.	G	A	intron	0.92
	OPRM1 118	OPRM1 118	6q24–q25	rs1799971	A	G	N40D	0.78
	OPRM1 440	OPRM1 50431	6q24–q25	rs # n.a.	C	G	S147C	0.84
	OPRM1 691	OPRM1 51325	6q24–q25	rs2075572	C	G	intron	0.54
Opioid receptor, delta 1 (OPRD1)	OPRD1 80	OPRD1 80	1p36.1–p34.3	rs1042114	T	G	C27F	0.91
	OPRD1 921	OPRD1 50702	1p36.1–p34.3	rs2234918	T	C	G307G	0.63
Opioid receptor, kappa 1 (OPRK1)	OPRK1 36	OPRK1 36	8q11.2	rs1051660	G	T	P12P	0.84
	OPRK1	OPRK1 10807	8q11.2	rs1365097	A	G	intron	0.69
	OPRK1	OPRK1 10915	8q11.2	rs1365098	G	T	intron	0.66
	OPRK1	OPRK1 11220	8q11.2	rs997917	A	G	intron	0.54
	OPRK1 459	OPRK1 16128	8q11.2	rs7815824	C	T	S153S	0.90
	OPRK1 843	OPRK1 21441	8q11.2	rs702764	A	G	A281A	0.72
	OPRK1 846	OPRK1 21444	8q11.2	rs # n.a.	C	T	V282V	0.97
Proopiomelanocortin (POMC)	POMC 18	POMC 18	2p23.3	rs8192605	C	T	C6C	0.99
	POMC 282	POMC 3170	2p23.3	rs # n.a.	C	T	S94S	0.92
	POMC 313	POMC 3201	2p23.3	rs # n.a.	G	T	E105Stop	0.96
	POMC 346	POMC 3234	2p23.3	rs # n.a.	C	T	L116L	0.98
	POMC 585	POMC 3473	2p23.3	rs2071345	C	T	A195A	0.94
	POMC 866	POMC 3755	2p23.3	rs1042571	C	T	3′-UTR	0.85
Proenkephalin (PENK)	PENK 28	PENK −588	8q23–q24	rs2609999	C	A	5′-UTR	0.57
	PENK 808	PENK 4686	8q23–q24	rs3839874	C	del	3′-UTR	0.67

db SNP rs # – accession number of SNP in NCBI dbSNP database; allele frequency is based on controls of this study. rs # n.a. – SNP is not listed in NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/).

a gene involved in the pathogenesis of emotional disorders, especially anxiety and panic disorders (Bowen et al., 1998; Geracioti et al., 1989; Hattori et al., 2002; Kennedy et al., 1999b). Opioid peptides are also implicated in the development of emotional disorders (Alda et al., 2000; Peckys and Hurd, 2001). As proopiomelanocortin (POMC) is a precursor for adrenocorticotropin hormone (ACTH) and patients with mood disorders have disturbances in the hypothalamic–pituitary–adrenal (HPA) system, POMC is a good target for association studies (Galard et al., 2002). 5-HT and DA are monoamines which are frequently studied in respect to mood disorders (Nutt, 2002; Pania and Gessab, 2002). Genes of the above described neurotransmitters and their receptors were chosen for genotyping. Altogether we analysed 91 polymorphisms located in 21 candidate genes (detailed information about the studied polymorphisms is available in Table 1). SNP analysis was performed by arrayed primer extension (APEX) technology. APEX is a genotyping and resequencing technology that combines Sanger dideoxy sequencing with the parallelization and high-throughput potential of microarray format (Tõnisson et al., 2002). APEX technology is suitable for SNP analysis allowing the screening of hundreds of SNPs in one sample.

Methods

Subjects and psychiatric assessment

Unrelated patients ($n = 177$; 39 male, 138 female; age range 18–73 yr; mean age 40.3 yr) with MDD were recruited in the study along with healthy control individuals ($n = 160$; 49 male, 111 female; age range 18–71 yr; mean age 37.7 yr) from the Estonian population. Diagnoses of patients were substantiated by psychiatric interview and verified by Mini International Neuropsychiatric Interview (M.I.N.I. 5.0.0) based on DSM-IV (Sheehan et al., 1998). The case group consisted of patients with only MDD ($n = 69$) and MDD patients with comorbid anxiety disorders [panic disorder, generalized anxiety disorder (GAD), obsessive–compulsive disorder (OCD), social phobia] ($n = 108$). Controls were evaluated using M.I.N.I. 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. Patients were recruited among consecutive outpatients and in-patients at the Clinic of Psychiatry of Tartu University Clinics and controls were recruited 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.

Template preparation and genotyping

Standard high-salt extraction method was used to isolate genomic DNA from 9 ml venous blood samples. Two different PCR programs were used to amplify the genomic regions containing the whole set of studied 91 polymorphisms with 64 individual PCR reactions. In program 1 amplification reactions consisted of an initial 5 min denaturation at 95 °C, followed by 34 cycles of: 95 °C for 30 s, 55 °C for 40 s, 72 °C for 40 s. The final extension step was 72 °C for 6 min. Program 2 contained temperature decrements of 1 °C per cycle in annealing step for first 10 cycles. Samples were processed in a PTC-200 thermal cycler (MJ Research Inc., Watertown, MA, USA). Primer sequences and PCR conditions used for amplification are available upon request.

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 Tõnisson et al. (2002). Production of oligonucleotide microchips and APEX reactions were performed as described earlier (Tõnisson et al., 2002). Slides were imaged with Genorama Quattroimager detector (Asper Biotech Ltd, Tartu, Estonia) and polymorphisms were identified by Genorama™ 4.1 genotyping software (Asper Biotech Ltd) by using signal patterns from a wild-type DNA sequence as the reference.

Selection of SNPs

By choosing missense SNPs for genotyping, we reasoned that at least some of them are probably causative mutations affecting function of the encoded protein associated with the underlying phenotype. We included common synonymous SNPs in our study under the assumption that silent SNPs, being in linkage disequilibrium (LD) with unknown functional polymorphism, can reveal an association with the actual disease-causing SNP(s). SNPs in regulatory sequences are thought to have the potential to control the level of gene expression, therefore, in some genes polymorphisms in 5′ or 3′ untranslated regions and intronic SNPs were included.

Table 2. Results of association analysis of 91 polymorphisms in major depressive disorder

SNP	Allele 1	Allele 2	Gene	Allelic P MDD	Allele 2 frequencies MDD	Allele 2 frequencies Controls
246	G	A	CCKAR	0.006	0.09	0.03
−2102	C	A	DRD1	0.008	0.02	0.07
−7054	C	A	DRD2	0.03	0.14	0.08
68	G	C	HTR2C	0.02	0.10	0.17

SNP, single-nucleotide polymorphism; MDD, major depressive disorder.

Statistical analysis

Association analysis statistics was performed using GENEPOP Version 3.3 software (Raymond and Rousset, 1995). 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 estimating simultaneously haplotype frequencies and haplotype–phenotype association as described in Tregouet et al. (2002). Pairwise LD was estimated by a log-linear model and the extent of disequilibrium was expressed in terms of standardized D' characteristic. Bonferroni correction was used after association and haplotype analysis to adjust for multiple testing.

Results

We genotyped 91 polymorphisms (87 SNPs and 4 insertions/deletions) in 21 candidate genes in 177 unrelated MDD patients and 160 healthy controls. In our screening set, genetic variations in altogether four genes displayed association with MDD. Data for statistically significant SNPs are presented in Table 2. Namely, SNPs 246G/A in CCKAR, −2102C/A in DRD1, −7054C/A in DRD2, and 68G/C (rs6318) in HTR2C genes were associated with MDD. In the case of CCKAR and DRD2 markers an excess of minor alleles in the affected group was found. In contrast, the minor alleles of DRD1 and HTR2C markers were more frequent in control subjects. After Bonferroni correction, none of the described marker-disease associations remained statistically significant. There was no deviation from Hardy–Weinberg equilibrium expectations at any of the genotyped loci. A gender comparison between females ($n=139$) and males ($n=38$) of the MDD sample did not show any significant differences with regard to alleles and/or genotypes. Our data indicate that the relationship between unipolar affective disorder and analysed loci appear to be independent of sex. Haplotype analysis was performed according to particular pairwise LD pattern for each gene (cases + controls, $n=337$). Only genes that were genotyped for two or more SNPs and showing the presence of LD in both affected and control groups, and having preliminary evidence of marker-disease association were included in haplotype analysis. It was also possible to investigate the effect of each SNP on different haplotypic background using the inference method. The odds ratio for MDD was estimated according to the haplotypic background conferred by other polymorphisms. Haplotype analysis revealed CCKAR haplotypes to be associated with MDD and altogether six haplotypes (HT) were found (Table 3). Reference haplotypes combined with the major alleles at each locus, which taken together with another common haplotype constituted almost 90% of all alleles. Both haplotypes were almost equally represented in cases and control subjects. Other haplotypes were rare. Haplotype 3 (GAGT) was significantly over-represented in the affected group, reflecting a higher frequency of the rare 246A allele in cases by comparison to the reference haplotype (GGGT). This haplotype (GAGT) was associated with a higher risk for MDD (OR 7.42, 95% CI 2.13–25.85, $p=0.002$) compared to the reference haplotype (GGGT). This haplotype effect also remained significant after Bonferroni correction ($p=0.04$ after Bonferroni's adjustment). We detected a significant individual SNP effect (OR 7.40, $p=0.002$) for 246G/A in a haplotype context HT1 (GGGT) vs. HT3 (GAGT). The test of a global CCKAR haplotypic association with MDD was significant in the population studied ($\chi^2=17.60$, d.f. $=5$, $p=0.004$).

Taken together, results of haplotype analysis confirmed our findings from the association study. Haplotype analysis revealed that CCKAR haplotype (GAGT) formed by SNPs at positions −128G/T (rs1800908), 246G/A, 608G/A (rs1800856), and 1266T/C (rs1800857) is a possible susceptibility haplotype for MDD.

Discussion

Clinical as well as molecular genetic studies indicate that MDD is a polygenic disorder. Many genes, each of minor individual contribution, are likely to be involved in the development of affective disorders. In our screening set of 91 polymorphisms in 21 candidate genes, variations in four genes displayed an association with MDD. Polymorphisms in CCKAR

Table 3. Estimated haplotype (HT) frequencies and HT effects in the CCKAR gene

HT	Single-nucleotide polymorphism				Haplotype frequency		Haplotypic OR (95% CI)	*p*
	−128G/T	246G/A	608G/A	1266T/C	Controls	Patients		
1	G	G	G	T	67.5	65.6	*	
2	G	G	G	C	21.6	20.0	0.905 (0.611–1.338)	0.625
3	G	A	G	T	1.2	7.5	7.418 (2.129–25.85)	0.002*
4	T	G	G	T	3.8	2.6	0.517 (0.203–1.320)	0.168
5	G	G	A	T	2.4	1.3	0.588 (0.137–2.523)	0.475
6	G	A	G	C	2.4	1.3	0.588 (0.137–2.523)	0.475

* $p = 0.04$ after Bonferroni's adjustment.

(246G/A), DRD1 (−2102C/A), DRD2 (−7054C/A), and HTR2C (68G/C, rs6318) genes were associated with MDD phenotypes.

Pharmacological studies have suggested that MDD is associated with impairment of brain monoaminergic transmission (Nemeroff, 2002). The role of 5-HT in the pathology of mood disorders is based mainly on the efficacy of selective 5-HT reuptake inhibitors in the treatment of MDD. DA has also been implicated in the pathophysiology of mood disorders and hypoactivity of the mesolimbic DA pathway may be related to depressive symptoms. Thus, genes that control the brain 5-HT and DA pathways seem to be good candidates for mediating genetic susceptibility to MDD.

Association of CCKAR gene polymorphism with MDD was further confirmed by haplotype analysis, where the GAGT haplotype carrying the risk for MDD (OR 7.418, $p = 0.002$) was established. CCKAR polymorphisms have been shown to be involved in schizophrenia and auditory hallucinations (Wang et al., 2002; Wei and Hemmings, 1999), and also in panic disorder (Miyasaka et al., 2004). Preclinical studies suggest that CCKAR directly regulates the release of DA in the nucleus accumbens and amygdala (Hamilton and Freeman, 1995). Therefore, CCKAR is implicated in the regulation of emotional behaviour and motivation. Supportive evidence of CCKAR gene involvement in mood disorders is also related to its genomic localization (4p15.1–p15.2). This locus is close to the 4p16 region which has been repeatedly shown to be related to bipolar disorder (Kennedy et al., 1999a). In our previous study we found that polymorphisms in the wolframin (WFS1) gene, also located in the 4p16 region, are possibly related to an increased risk for mood disorders (Koido et al., 2004). This study sample was partially the same as in the present study. Als and colleagues found that markers in the 4p15 region appeared to be associated with schizophrenia and schizophrenia combined with bipolar disorder, and also supportive evidence for schizophrenia and bipolar disorder being associated with the 4p16 region (Als et al., 2004). Therefore, the 4p15–p16 region seems to be a good candidate risk locus for psychiatric disorders.

Results of this study provide further evidence for the involvement of genes related to monoaminergic and peptidergic neurotransmission in the regulation of mood disorders. However, we cannot exclude a hypothesis describing polymorphisms as being in LD with other functionally significant polymorphisms, which could actually be involved in mood disorders. It has been shown that missense SNP itself probably does not cause disease but it is in strong LD with non-functional SNP which may actually contribute to the susceptibility for disease (Handoko et al., 2004). This warrants studying not only functional polymorphisms but also untranslated SNPs.

Due to the limited size of our sample this study should be considered an exploratory in nature. A multi-stage approach is recommended to distinguish false-positive discoveries from real associations (Hirschhorn and Daly, 2005). As many association studies produce unreplicable results due to false-positive findings induced by multiple testing, it is suggested that first, many markers should be typed for a subset of individuals. Afterwards the most promising markers can be evaluated on a larger sample (van den Oord and Sullivan, 2003). Therefore, replication studies with larger and independent samples are needed.

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Statement of Interest

A. Metspalu is a scientific advisor and member of the Council of Asper Ltd.

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