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Brief Research Communication

Investigating the Role of *p11* (*S100A10*) Sequence Variation in Susceptibility to Major Depression

Ranjana Verma,[1] David J. Cutler,[2] Peter Holmans,[3] James A. Knowles,[4] Raymond R. Crowe,[5] William A. Scheftner,[6] Myrna M. Weissman,[7] J. Raymond DePaulo Jr.,[1] Douglas F. Levinson,[8] and James B. Potash[1]*

[1]*Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, Maryland*
[2]*McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland*
[3]*Biostatistics and Bioinformatics Unit, Wales College of Medicine, Cardiff University, Cardiff, United Kingdom*
[4]*Zilkha Neurogenetic Institute, Keck School of Medicine, University of Southern California, Los Angeles, California*
[5]*Department of Psychiatry and Mental Health Clinical Research Center, University of Iowa Carver College of Medicine, Iowa City, Iowa*
[6]*Department of Psychiatry, Rush-Presbyterian Medical Center, Chicago, Illinois*
[7]*Department of Psychiatry, College of Physicians and Surgeons, Columbia University, and the New York State Psychiatric Institute, New York*
[8]*Department of Psychiatry, Stanford University School of Medicine, Palo Alto, California*

Recent evidence suggests a potential role for the *p11* gene in conferring risk to depressive disorders. *p11* has been shown to influence serotonergic transmission, and its expression was found to be reduced in a mouse model of depression, as well as in post-mortem brain tissue from major depressive disorder (MDD) cases. In the present study, we tested for rare variants in *p11* by resequencing promoter, exonic and flanking intronic regions in 176 MDD cases and 176 matched controls. We also assessed common variation by genotyping eight single nucleotide polymorphisms (SNPs), seven tag SNPs and one found through resequencing, in 641 cases and 650 controls. Resequencing revealed nine novel rare variants, including a missense mutation (Asp60Glu) observed in one case and one control, and four variants that occurred only in cases and not controls. The number of rare variants in cases did not exceed that expected by chance for the length of sequence analyzed, and also was not significantly greater than that observed in controls. Resequencing also identified two known SNPs, one (rs4845720) of which was significantly more frequent in cases than controls in the resequenced sample (3.1% vs. 0.9%, $P = 0.03$), though not in the larger sample (3% vs. 2%, $P = 0.15$). None of the tag SNPs showed any evidence of association. Our results do not support a major role for either common or rare *p11* SNPs with MDD. Several limitations of the study are discussed.

KEY WORDS: *p11*; S100A10; serotonin; tag SNPs; association; major depression

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Family, twin and adoption studies have suggested that genetic factors contribute substantially to the etiology of major depressive disorder (MDD), particularly with recurrent episodes and early age at onset, but as yet no susceptibility gene has been definitively implicated by association studies [Levinson, 2006].

Recently, *p11* has emerged as a potential functional candidate gene for MDD [Svenningsson et al., 2006]. It was shown to be involved in serotonergic transmission, interacting with 5-HT1B receptors, and increasing their localization at the cell surface. Decreased levels of *p11* mRNA and protein were observed in a mouse model of depressive illness, and in post-mortem brain tissue from MDD patients; expression was increased by antidepressants and electroconvulsive therapy; and over-expressing the gene evoked behaviors similar to those induced by antidepressants.

The S-100 protein family, including *p11* (or *S100A10*), is characterized by common structural motifs including two EF-hand calcium binding domains, but in *p11* both calcium binding sites are inactive [Donato, 2001]. The *p11* gene, on chromosome 1q21.3, spans ~11 kb with three exons encoding a protein of 97 amino acids [Harder et al., 1992]. It has a well-characterized promoter sequence with several experimentally demonstrated binding sites for factors that regulate gene transcription [Huang et al., 2003].

Using a large cohort of recurrent, early-onset MDD cases and controls, we (1) searched for novel variants in *p11* by resequencing a sub-sample selected for within-family 1q21.3 allele sharing; (2) tested whether rare (unique) variants were more frequent in cases; and (3) evaluated possible association of MDD with common tag single nucleotide polymorphisms (SNPs) in the entire sample set.

We studied cases from 641 European-ancestry families recruited by the Genetics of Recurrent Early Onset Depression (GenRED) study [Levinson et al., 2003]. MDD probands had ≥2 lifetime episodes (or chronic MDD), onset before age 31, and

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*Correspondence to: James B. Potash, Johns Hopkins Hospital, 600 North Wolfe Street, Meyer 4-119, Baltimore, MD 21287-7419. E-mail: jpotash@jhmi.edu

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TABLE I. Frequency Distribution of *p11* Sequence Variations in Cases and Controls Identified Through Resequencing*

Position[a]	Variant (major/minor allele)	Location	Novel/dbSNP	Minor allele n (frequency)	
				Cases	Controls
150234300	A/T	Promoter[b]	rs4845720	11 (0.031)	3 (0.009)
150233568	C/T	Promoter[b]	Novel	1 (0.003)	0 (0)
150232972	A/G	Exon 1	rs11542015	149 (0.431)	137 (0.398)
150232944	G/A	Exon 1	Novel	2 (0.006)	2 (0.006)
150232926	A/G	Exon 1	Novel	1 (0.003)	0 (0)
150232878	C/T	Exon 1	Novel	0 (0)	1 (0.003)
150232872	(—/tcgcccagc)	Exon 1	Novel	12 (0.034)	18 (0.051)
150232871	T/G	Exon 1	Novel	1 (0.003)	0 (0)
150225355	A/G	Intron 1	Novel	1 (0.003)	0 (0)
150222427	(—/T)	Intron 2	Novel	9 (0.026)	8 (0.023)
150222377	C/G (Asp-Glu)	Exon 3	Novel	1 (0.003)	1 (0.003)

*A total of eleven sequence variations were identified in the *p11* gene with four novel variants (highlighted in gray) observed only in cases and not in controls. The dbSNP rs4845720 (underlined) showed modest association with MDD ($P = 0.03$).
[a]Position of variants is in reference to NM_002966 (UCSC March 2006).
[b]Locations of promoter region SNPs (at 150234300 and 150233568) in relation to the transcription start site in NM_002966 (UCSC March 2006) are −932 and −230, respectively.

≥1 sibling with MDD (onset before age 41). All subjects gave written informed consent based on protocols approved by local Institutional Review Boards. The case-control sample included 641 MDD probands and 650 European-ancestry controls (NIMH Center for Genetic Studies, http://nimhgenetics.org/). Controls were selected for absence of recurrent MDD with onset before age 41 by self-report questionnaire based on the CIDI-Short Form [Kessler et al., 1998]. For resequencing, 176 GenRED families were identified who showed the greatest evidence for linkage on 1q21.3 in a genome-wide scan [Holmans et al., 2007], and then the case within each family was selected who demonstrated the highest IBD sharing with affected relatives [Fingerlin et al., 2004] (the proband was selected in 96 families, while a non-proband affected case was selected in 80 additional families). The 176 resequenced controls were selected randomly.

To identify novel variations in *p11*, we resequenced nearly 3.2 kb, including 1.5 kb of the promoter, all exons and flanking intronic regions, using seven primer sets (primers and reaction conditions available on request) on an Applied Biosystems 3100 Genetic Analyzer. Sequences were aligned with NCBI database reference sequences (Build 36.1) using the Vector NTI Advance™ 10 multiple alignment tool.

To assess common sequence variation, we selected seven tag SNPs covering the gene and extending 3 kb flanking the untranslated regions, from European HapMap II data, using the pair wise algorithm of TAGGER [de Bakker et al., 2005] to select tags with minor allele frequencies ≥0.05 and a linkage disequilibrium parameter of $r^2 = 0.8$. The same seven tag SNPs were selected with an LD parameter of $r^2 = 1.0$, indicating full coverage of HapMap II variation. The tag SNPs and one promoter SNP (rs4845720—nominally associated in sequencing samples) were genotyped in the entire sample set using the Taqman Allelic Discrimination method and an Applied Biosystems 7900 HT Sequence Detection System, with call rates exceeding 99%. We checked duplicates and family samples on each genotyping plate as quality controls.

We considered the possible significance of rare variants in three ways. As suggested by Cohen et al. [2004], we were prepared to contrast the number of rare non-synonymous variants observed in only cases versus those found only in controls, but none were found. While it is more speculative to hypothesize a role for other rare variants, we also examined this possibility by using a population genetics-based method [Mitchell et al., 2005] to determine whether there were more rare (<1%) variants observed only in cases than would be expected in this length of sequence, and by contrasting the numbers of rare variants observed only in cases and only in controls. We tested for deviation from Hardy–Weinberg equilibrium as a quality check. Allelic and genotypic case-control association were tested with the chi-square statistic. We employed a modification of EATDT [Lin et al., 2004] for case-control data to exhaustively test all possible haplotype combinations.

This study had over 80% power to detect (at permutation-corrected $P < 0.05$, corresponding to nominal $P = 0.0075$) an allele or haplotype conferring a genotypic relative risk of at least 1.3–1.7, depending on the risk allele frequency, across a broad range of frequencies (0.1–0.9).

Resequencing of the *p11* gene revealed nine novel heterozygous variations including six exonic SNPs, two intronic SNPs, and one SNP in the promoter region. All novel variants, including two insertion/deletion variations and seven substitutions, were rare (Table I). The novel exonic variants included a missense mutation (Asp60Glu) in exon 3 identified in one case and one control. Four novel sequence variants were identified only in cases and not in controls ($P = 0.225$ for likelihood of this many rare variants in cases as compared to the number expected by chance in the given length of region sequenced). One additional novel variant was found only in one control ($P = 0.188$ for the difference in the frequency of novel variants in cases vs. controls). We also observed two dbSNPs (rs4845720 and rs11542015) located within the promoter and exon 1, respectively. The minor allele for rs4845720 (located 20 bp upstream of a CTF/NF1 transcription factor binding site) was present in the heterozygous state in 11 cases (3.1%) and 3 controls (0.9%) ($P = 0.03$). The minor allele for rs11542015 was present in nearly equal frequencies in cases (N = 149, 43%) and controls (N = 137, 40%) (Table I).

All genotyped SNPs were in Hardy-Weinberg equilibrium in both cases and controls. None of the tag SNPs showed any allelic or genotypic association with MDD in the case-control analysis (Table II). Exhaustive haplotypic analysis revealed 38 unique haplotypes, with the best permutation-corrected $P = 0.27$. Because the non-tag SNP rs4845720 showed nominally significant case-control differences in the sequencing sample, it was studied in the larger sample of 641 cases and 650 controls. The minor allele was seen in 36 cases (3%) and

TABLE II. Frequency Distribution of *p11* tag SNPs and rs4845720 in 641 Cases and 650 Controls

dbSNPID	Position[a]	Variant	Location	Sample type	Genotype n (frequency)[b]			Allele n (frequency)[b]	
					Homozygous major allele	Heterozygous	Homozygous minor allele	Major allele	Minor allele
rs4845720	150234300	A/T	Promoter	Cases	602 (0.94)	36 (0.06)	0 (0)	1,240 (0.97)	36 (0.03)
				Controls	615 (0.96)	25 (0.04)	0 (0)	1,255 (0.98)	25 (0.02)
rs1038745	150233369	C/A	Promoter	Cases	596 (0.94)	35 (0.06)	0 (0)	1,227 (0.97)	35 (0.03)
				Controls	623 (0.96)	25 (0.04)	0 (0)	1,271 (0.98)	25 (0.02)
rs1552607	150230022	C/G	Intron 1	Cases	507 (0.82)	107 (0.17)	5 (0.01)	1,121 (0.91)	117 (0.09)
				Controls	546 (0.85)	94 (0.14)	4 (0.01)	1,186 (0.92)	102 (0.08)
rs1873311	150228846	A/G	Intron 1	Cases	523 (0.82)	108 (0.17)	6 (0.01)	1,154 (0.91)	120 (0.09)
				Controls	542 (0.84)	95 (0.15)	3 (0.01)	1,179 (0.92)	101 (0.08)
rs11204922	150228199	A/G	Intron 1	Cases	249 (0.39)	296 (0.47)	90 (0.14)	794 (0.63)	476 (0.37)
				Controls	261 (0.40)	282 (0.44)	103 (0.16)	804 (0.62)	488 (0.38)
rs3791153	150224768	T/C	Intron 2	Cases	295 (0.47)	271 (0.43)	67 (0.10)	861 (0.68)	405 (0.32)
				Controls	286 (0.44)	291 (0.45)	68 (0.11)	863 (0.67)	427 (0.33)
rs2338019	150223438	A/G	Intron 2	Cases	199 (0.31)	312 (0.49)	125 (0.20)	710 (0.56)	562 (0.44)
				Controls	210 (0.32)	317 (0.49)	120 (0.19)	737 (0.57)	557 (0.43)
rs6587640	150221854	G/A	Locus-region	Cases	275 (0.43)	277 (0.44)	82 (0.13)	827 (0.65)	441 (0.35)
				Controls	271 (0.42)	294 (0.46)	79 (0.12)	836 (0.65)	452 (0.35)

[a]Position of variants is in reference to NM_002966 (UCSC March 2006).
[b]No allelic or genotypic differences were statistically significant for either the tag SNPs or rs4845720, the SNP (highlighted in gray) that showed modest association in the smaller sequencing sample set.

25 controls (2%) ($P = 0.15$). Given the substantial power of the study to detect association with common alleles, it is unlikely that any common SNP in *p11* confers a genotypic relative risk of 1.3–1.7 or greater.

Our results do not support a major role for *p11* sequence variation in MDD susceptibility. However, several limitations should be considered. Rare variation was not studied in the introns (spanning nearly 75% of the gene) nor in the flanking regions; these regions could harbor variants that influence *p11* expression. There could be case-control differences in the frequency of very rare variants in the regions that were studied (i.e., very small differences requiring a larger sample to detect), in as-yet undetected common intronic variants that are not highly correlated with HapMap II SNPs, in common variants conferring very modest increases in illness risk (GRR <1.3–1.7), or in copy-number polymorphisms. A true association might be missed due to population stratification (e.g., if an ethnic group with a high frequency of a risk allele was underrepresented among cases); this is unlikely here given the very similar self-reported ancestries of our cases and controls (Supplemental Table I), but future association studies will benefit from the anticipated development of ancestry-informative marker sets for European subpopulations to enable formal tests of stratification. Finally, we cannot rule out the possibility that we detected a true but small association signal in our resequencing cases, who were selected because of evidence for allele sharing on chromosome 1q. Such an effect, if real, might be detected in a much larger sample.

The promoter SNP rs4845720 is located 20 bp upstream of a CTF/NF1 transcription factor binding site. CTF/NF1 has been shown experimentally to bind to the promoter of *p11* at −932 [Huang et al., 2003], though its influence on expression levels is not known. Neither is it known whether rs4845720, located near the CTF/NF1 site, influences binding or expression.

If variation in and near the *p11* gene does not confer vulnerability to MDD, it is possible that sequence variations in other interacting genes, located elsewhere in the genome, might increase the risk of MDD by influencing *p11* expression.

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