Published in final edited form as:
J Affect Disord. 2009 April ; 114(1-3): 279–285. doi:10.1016/j.jad.2008.08.005.

A missense variant (P10L) of the melanopsin (Opn4) gene is associated with Seasonal Affective Disorder

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Abstract

Background—Melanopsin, a non-visual photopigment, may play a role in aberrant responses to low winter light levels in Seasonal Affective Disorder (SAD). We hypothesized that functional sequence variation in the melanopsin gene (Opn4) could contribute to increasing the light needed for normal functioning during winter in SAD.

Methods—Associations between alleles, genotypes, and haplotypes of Opn4 in SAD participants (n = 130) were performed relative to controls with no history of psychopathology (n = 90).

Results—SAD participants had a higher frequency of the homozygous minor genotype (T/T) for the missense variant rs2675703 (P10L) than controls, compared to the combined frequencies of C/C and C/T. Individuals with the T/T genotype were 5.6 times more likely to be in the SAD group than the control group.

Limitations—The study examined only one molecular component of the non-visual light input pathway, and recruitment methods for the comparison groups differed.

Conclusion—These findings support the hypothesis that melanopsin variants may predispose some individuals to SAD. Characterizing the genetic basis for deficits in the non-visual light input pathway has the potential to define mechanisms underlying the pathological response to light in SAD, which may improve treatment.

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The opinions and assertions expressed herein are those of the authors and are not to be construed as expressing the views of the USUHS or the United States Department of Defense.

Conflict of Interest

All authors declare that they have no conflicts of interest.

*Contributors

Author Roecklein designed the study, wrote the protocol, managed the literature searches and analyses, undertook the statistical analysis, and wrote the first draft of the manuscript. Author Rohan supervised the aforementioned activities. Author Duncan managed the data stored at the NIH pertaining to this sample. Author Lipsky designed the protocol and laboratory methods. Author Provencio supervised all activities including manuscript preparation and laboratory methods. Author Rosenthal oversaw the sample collection, clinical assessment, and diagnosis. All authors contributed to and have approved the final manuscript.

Keywords

Melanopsin; seasonal affective disorder; Opn4

1. Introduction

Seasonal affective disorder (SAD) is characterized by recurrent depressions in fall and winter (Rosenthal et al., 1984a). Aberrant responses to light in SAD are suggested by the seasonality of depressive episodes, the favorable antidepressant response to light therapy (Golden et al., 2005), and a lengthened melatonin release profile in winter (Wehr et al., 2001), and may be mediated by abnormal retinal phototransduction (Wehr et al., 1987).

Twin studies measuring seasonal variation in mood and behavior indicate that 29%–69% of seasonality is heritable (Jang et al., 1997; Madden et al., 1996). Most candidate gene studies for SAD have focused on the serotonin transporter (Johansson et al., 2001; Johansson et al., 2003a; Rosenthal et al., 1998; Sher et al., 2000; Thierry et al., 2004; Willeit et al., 2003), the serotonin receptor 2A (Enoch et al., 1999; Johansson et al., 2001; Lee et al., 2006), the D4 dopamine receptor (Levitan et al., 2004, a, b), and circadian clock genes (Johansson et al., 2003b; Paik et al., 2007; Partonen et al., 2007). Positive findings in these studies account for only a small proportion of the esitmated heritability of SAD.

We hypothesize that the light input pathway that mediates non-visual functions may modulate the symptoms of SAD through genetic variations in molecular components of this pathway. A key signaling component of the human non-visual light input pathway, the photopigment melanopsin (Provencio et al., 2000; Provencio et al., 2002; Rollag et al., 2003), is found in retinal ganglion cells projecting to non-visual centers of the brain including the suprachiasmatic nucleus (Hattar et al., 2006). Intrinsically photosensitive, melanopsin-containing retinal ganglion cells have been implicated in functions including circadian photoentrainment (Hattar et al., 2003; Panda et al., 2003; Panda et al., 2002; Ruby et al., 2002), negative masking (Mrosovsky and Hattar, 2003), and the pupillary light reflex (Hattar et al., 2003; Lucas et al., 2003; Panda et al., 2003). It is possible that sequence variations in the melanopsin gene may affect light input to the brain, increasing vulnerability to SAD. The present study uses a candidate gene approach to investigate the potential involvement of haplotypes in the melanopsin gene, in addition to specific single nucleotide polymorphisms (SNPs) that result in coding variants of the melanopsin protein.

2. Methods

SAD Participants volunteered for SAD research at the National Institute of Mental Health in response to community advertising. Inclusion criteria for the SAD group included a mood disorder with seasonal pattern, and no history of other Axis I disorders established using the Structured Clinical Interview for DSM-III Axis I Disorders (SCID; Spitzer et al., 1990). Control participants were recruited from the NIH Healthy Volunteers Program through which individuals can volunteer for many types of studies. Controls had no personal or family history of any Axis I disorder and had low levels of seasonality, as measured by the Seasonal Pattern Assessment Questionnaire (SPAQ; Rosenthal et al., 1984b). Seasonal variation in six SPAQ items is combined to form the Global Seasonality Score (GSS; an index of seasonal variations in sleep, appetite, mood, energy level, weight and social behavior).

SNPs were densely spaced throughout the gene and proximal regions (Table 1). SNPs were identified through the NCBI dbSNP public database (Sherry et al., 2001). Missense variants

identified in the Opn4 gene included in the study were: rs2675703 (P10L), rs1079610 (I394T), and rs12262894 (D444G).

Regions of interest in Opn4 were amplified using Polymerase Chain Reaction (PCR) with the primer pairs reported in Table 1. SNP genotyping utilized 5′-exonuclease assays with allele-specific fluorescence detection probes (Table 1), developed with Assays-on-Demand (Applied Biosystems, Foster City, CA). Missense variations were genotyped by direct sequencing of PCR products using ABI 2.0 Big Dye Sequencing chemistry. A total of 231 samples were available, however <3% contained DNA at concentrations too low to yield sequence. Failure and error rates (<10%) are listed in Table 1.

Prior to tests of association, haplotypes were constructed using multiple iterations of haplotype inference with the application PHASE 2.1 (Stephens et al., 2001). The haplotype estimation was performed twice, omitting haplotypes with frequency <3%, yielding six haplotypes. Cases and controls did not deviate from Hardy Weinberg Equilibrium (HWE) on the basis of allele or genotype frequency. With the available sample size of $N = 222$, 80% power existed to detect an effect with an odds ratio of 1.65.

Logistic regression was used to test for associations between group (SAD or control), haplotype, genotype, and allele frequency with demographic variables (age, gender, and ethnicity) as covariates. Within SAD participants, analysis of covariance was used to test for an association between GSS and genotypes, alleles, and haplotypes, while controlling for potential interactions with gender, age, type of SAD (i.e., unipolar, Bipolar I, or Bipolar II), and race.

3. Results

Participants in the SAD group were 70% female, 95% Caucasian, 5% African American, and 1% Asian American, and middle aged ($M = 40.1$ years, $SD = 8.3$). They had an average GSS of 16.6 ($SD = 3.3$, min = 8, max = 24). Most participants (53%) had a diagnosis of unipolar Major Depressive Disorder, whereas 17% had a diagnosis of Biploar I Disorder, and 2% had Bipolar II Disorder (28% missing data). Among individuals with SAD, differences in GSS between the mood disorder diagnostic groups (i.e., unipolar, Bipolar I and II) were not statistically significant.

Participants in the control group were 58% female, 97% Caucasian, 3% African American, and middle aged ($M = 39.8$ years, $SD = 10.7$). Controls had a maximum GSS of 10 ($M = 2.23$, $SD = 2.01$). The SAD and control groups were not significantly different on the basis of age [$F(1, N = 228) = 0.063$, *ns*], gender [$X^2(1, N = 228) = 3.45$, *ns*], or ethnicity [$X^2(2, N = 228) = 1.108$, *ns*].

3.1 Allele and Genotype Association Tests

The distribution of all three genotypes (C/C, C/T, and T/T) and both alleles between the SAD and control groups at rs2675703 (P10L) did not differ [Genotype: Fisher's Exact Test ($2, N = 228$) = 5.09, $p = .09$, *ns*; Allele: Fisher's Exact Test ($1, N = 456$) = 2.29, *ns*]. Upon observing that all seven individuals with the T/T genotype at P10L were in the SAD group, an autosomal recessive disorder interpretation was considered. If this were the case with P10L and SAD, the homozygous T/T genotype would be more common in SAD patients than in controls. A 2×2 test indicated SAD participants had a higher frequency of T/T than controls, when compared to the combined frequency of C/C and C/T together, Fisher's Exact test ($1, N = 220$) = 4.38, $p < .05$. Only 7 (5%) SAD participants had the T/T genotype compared to zero controls (Table 2). The effect size for this finding is medium, $d = 0.46$, computed using the Arcsine test (Lipsey and Wilson, 2001). The Odds Ratio, $OR = 5.63$ [95% CI 1.22–26.01], indicated that individuals

with the T/T genotype were 5.6 times more likely to be in the SAD group than in the control group. Demographic variables did not account for variance in this associated when tested using logistic regression; Genotype: *OR* = 1.553 (95% *CI* 0.90 – 2.68), *ns*; Allele: *OR* = 1.67 (95% *CI* 0.94 – 2.97), *ns*.

The groups did not differ on frequency of genotypes and alleles at the I394T locus; Genotype: Fisher's Exact Test (2, *N* = 217) = 1.50, *ns*, Allele: Fisher's Exact Test (1, *N* = 434) = 0.668, *ns*. In addition, I394T genotype and allelic frequency did not predict group membership in logistic regression; Genotype: *OR* = 0.93 [95% *CI* 0.64 – 1.37], *ns*; Allele: *OR* = 0.805 (95% *CI* 0.53 – 1.22), *ns*.

The D444G locus in the melanopsin gene was found to be monomorphic in the present sample, with all participants having the homozygous genotype for the major allele (C). This is consistent with data reporting no polymorphisms at this site in Caucasian samples, and very low rates in non-Caucasian samples, accessed through online databases (dbSNP; Build 120/126).

The association between P10L genotype and GSS, with age, gender, race, and diagnosis (i.e., unipolar, Bipolar I, or Bipolar II) as covariates among SAD patients was nonsignificant, $F(2, 94) = 1.33$, *ns*. There was no association between I394T genotype and GSS among SAD patients, $F(2, 86) = 1.75$, *ns.*

3.2 Haplotype Analyses

Pairwise rates of linkage disequilibrium calculated with HaploView (Barrett et al., 2005) identified Logarithm of the Odds Ratio's greater than 2 and $D' = 1$ for each pair of markers, indicating the melanopsin gene exists as one haplotype block. Individuals with rare haplotypes (i.e., <2% frequency) were removed from further analyses as likely genotyping errors, yielding 190 individuals for the final haplotype analyses. Of the rare haplotypes deleted from the final analyses, the majority (70%) had neither the P10L or I394T coding variant, 8% had the P10L variant, 20% had the I394T variant, and 2% had both. Therefore, it is unlikely that potentially important haplotypes were removed.

There was no significant difference in haplotype distribution between the SAD and control groups, Fisher's Exact Test (5, 378) = 3.75, *ns*. None of the covariates or the frequency of the 6 haplotypes predicted group membership (i.e., SAD or control) when tested with logistic regression controlling for demograpic variables. No association was found between haplotype frequencies and GSS among SAD patients.

4. Discussion

These results suggest that melanopsin gene variants may increase risk for SAD in 5% of individuals with SAD. Allele, genotype, and haplotype-based analyses did not support an association between melanopsin gene variants and SAD in this study. However, a single missense variant (P10L) was associated with increased risk of SAD, specifically for individuals homozygous for the minor T allele. The effect size for this comparison is medium, larger than the small effect size expected for any one gene in complex disorders like SAD. All individuals with the T/T genotype were in the SAD group. Individuals with the T/T genotype were 5.6 times more likely to be in the SAD than in the control group.

With fewer than 2K bases between SNPs, a thorough analysis of the melanopsin gene was conducted. It is unlikely that the present study failed to assay important parts of the melanopsin gene within the known coding regions. It is most likely that regulatory regions further away from the gene are responsible for additional heritable risk for SAD related to melanopsin. A

limitation of the study is that the control and SAD participants were recruited using different methods.

Even though P10L may contribute to the development of SAD in only 5% of cases, these individuals may represent a subphenotype in what may turn out to be a heterogeneous condition. Identifying genetic risk factors for SAD might help identify new treatments, or predict which individuals are most likely to respond to currently available treatments.

Acknowledgments

Funding for this study was provided by NIMH Grant 5-F31-MH072054-02 (K. Roecklein), NIH grant RO1-NS052112-05 (I. Provencio), NIMH intramural award 92-M-0224 (N. Rosenthal), and NIAAA intramural award Z01-AA00325 (R. Lipsky).

Funding for this study was provided by NIMH Grant 5-F31-MH072054-02 (K. Roecklein), NIH grant RO1-NS052112-05 (I. Provencio), NIMH intramural award 92-M-0224 (N. Rosenthal), and NIAAA intramural award Z01-AA00325 (R. Lipsky); the NIH had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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Table 1

Single Nucleotide Polymorphisms Markers, PCR Primers, and Probes

RefSNP [a]	Location[b]	bp gap[c]	PCR Primers (F: Forward; R: Reverse)	MGB® Probes (3′ to 5′) [d]	Failure Rate	Error Rate
rs2675693	flanking 5′	3118	F: 3′-AAGAAAGACCTCAGGCCCCTAT-5′ R: 3′-CCTGAGCCTCAGTTTCTCCAA-5′	Fam: 3′-AGGCAAGGCCGGGG-5′ Vic: 3′-AGATAGATCAAAGCCCA-5′	<1%	0%
rs2675703 (P10L)[e]	Exon 1	1	F: 3′-AGGAAAGTTGGGAGGCTGAG-5′ R: 3′-GGTCAGGGAAGGCTCTGTG-5′	n/a	7.6%	0%
rs11202106	Exon 1	3154	(Sequenced with rs2675703/P10L primers)	n/a	4.4%	0%
rs10788521	intron (2)	1917	F: 3′-CACGTGTGTGCACATGCATAC-5′ R: 3′-TCCTGACACCTCCAGAGCTATG-5′	Fam: 3′-TGAGGGGTGCGGGA-5′ Vic: 3′-TGAGGGGTGTGGGAA-5′	<1%	0%
rs3740335	intron (5)	2475	F: 3′-GCTGACTGCCACCCGACTA-5′ R: 3′-AAGGTCTGGAGAGCCCTAGGA-5′	Fam: 3′-TGGACGATGCGTCCT-5′ Vic: 3′-TGGACGATGCATCCT-5′	<1%	0%
rs1079610 (I394T) [e]	Exon 8	1376	F: 3′-TGGCTTCTTCCCCCAGTGTA-5′ R: 3′-ATGTGCTTGGTGTGCATTCAG-5′	Fam: 3′-CTCCACGCTGATCAG-5′ Vic: 3′-CTCCACGCTGACCAG-5′	4.9%	9%
rs12262894 (D444G) [e]	Exon 9	2550	F: 3′-TGCCCAGCAAGCAAATGG-5′ R: 3′-GGTGCCTTGGCTTCCAAGTC-5′	Fam: 3′-TACGGTCAGGATCTG-5′ Vic: 3′-TACGGTCAGGGTCTG-5′	<1%	0%
rs3740341	3′UTR	2293	F: 3′-TGGCTTCTTCCCCCAGTGTA-5′ R: 3′-ATGTGCTTGGTGTGCATTCAG-5′	Fam: 3′-TCTTGTGCACGCGGG-5′ Vic: 3′-TCTTGTGCACACGGG-5′	<1%	0%
rs2803558	3′flanking	n/a	F: 3′-CAGCTATTCTTAGGAGCCTCTCAAGAG-5′ R: 3′-AAAGGGTAGAGAGGGTTGAGTGAGA-5′	Fam: 3′-AGGCAAGGCCGGGG-5′ Vic: 3′-AGGCAAGGCTGGGG-5′	<1%	0%

[a] RefSNP ID number is a unique code assigned to each polymorphism.

[b] Location; flanking 5′ region is before the gene, exons are coding regions of the gene, introns are non-coding regions of the gene between two exons, UTR is an un-translated region at the end of the gene, and 3′ flanking region is after the gene.

[c] bp to next SNP is the number of base pairs between the current marker and the next marker along the gene.

[d] Fam & Vic refer to dyes employed by the 5′-exonuclease assay, chemistry available through ABI (Foster City, CA).

[e] Coding variants include P10L, I394T, and D444G, while the remaining SNPs used in the analysis are noncoding variant.

Table 2

Frequency of genotype and alleles at the P10L & I394T loci.

	Genotype frequency *n* (%)				Allelic frequency *n* (%)	
P10L Locus	**C/C**	**C/T**	**P10L T/T**	**C/C and C/T**	**C**	**T**
SAD (*n* = 132)[b]	96 (73%)	29 (22%)	7 (5%)[a]	125 (95%)	221 (86%)	36 (14%)
Control (*n* = 90)	70 (78%)	20 (22%)	0 (0%)	90 (100%)	160 (89%)	20 (11%)
I394T Locus	C/C	C/T	I394T T/T	C/C and C/T	C	T
SAD (*n* = 128)	16 (13%)	56 (44%)	56 (44%)	72 (56%)	88 (34%)	168 (66%)
Control (*n* = 89)	11 (12%)	46 (52%)	32 (36%)	57 (64%)	68 (38%)	110 (62%)

[a] $p < .05$

[b] n's are different in each analysis due to differential success rates between the genotyping assays used for P10L vs. I394T.