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Association of polymorphisms in *HCN4* with mood disorders and obsessive compulsive disorder

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ABSTRACT

Hyperpolarization activated cyclic nucleotide-gated (HCN) potassium channels are implicated in the control of neuronal excitability and are expressed widely in the brain. HCN4 is expressed in brain regions relevant to mood and anxiety disorders including specific thalamic nuclei, the basolateral amygdala, and the midbrain dopamine system. We therefore examined the association of *HCN4* with a group of mood and anxiety disorders. We genotyped nine tag SNPs in the *HCN4* gene using Sequenom iPLEX Gold technology in 285 Caucasian patients with DSM-IV mood disorders and/or obsessive compulsive disorder and 384 Caucasian controls. *HCN4* polymorphisms were analyzed using single marker and haplotype-based association methods. Three SNPs showed nominal association in our population (rs12905211, rs3859014, rs498005). SNP rs12905211 maintained significance after Bonferroni correction, with allele T and haplotype CTC overrepresented in cases. These findings suggest *HCN4* as a genetic susceptibility factor for mood and anxiety disorders; however, these results will require replication using a larger sample.

Psychiatric disorders arise through the interplay of genetic and environmental risk factors [17]. Mood and anxiety disorders are highly comorbid [2,7,25,49] and show substantial shared genetic variance based on twin and family studies [11,23,24,31,36,51]. Therefore, there are likely to be genetic risk factors that determine risk for both classes of disorders jointly.

Hyperpolarization activated cyclic nucleotide-gated (HCN) ion channels underlie the hyperpolarization-activated current, I_h. HCN channels, coded by HCN1–4, are composed of four channel subunits [9] and modulate intrinsic neuronal excitability and synaptic integration [13,32–34,56]. The open probability of these channels is increased by cyclic adenosine monophosphate (cAMP) [4,9,19], making these channels highly susceptible to regulation by receptors coupled to cAMP. Of the four cloned HCN subunits, HCN4 is the most sensitive to cAMP [8,9].

There are numerous reasons to believe that HCN4 may be involved in mood and anxiety disorders. It has a key role in regulating the functioning of the thalamus, amygdala, mid-brain dopamine system, and indirectly the prefrontal cortex (PFC). HCN4 is highly expressed in the thalamus, including the paraventricular nucleus (PVT) [38], the ventrobasal complex, and the reticular thalamic nucleus (RTN) [1]. Lesions in these thalamic nuclei induce symptoms similar to PFC dysfunction, including impairment of executive function, initiative, and attention [52], suggesting the thalamic nuclei and their cortical targets can act as functional units. Abnormalities in thalamic regions have been described in mood disorders [10,22] and OCD [18,20], based on *post-mortem* [5,58] and *in vivo* anatomical and functional imaging techniques [14,15]. Orexin inhibits HCN currents [29] and produces anxiety-like responses in rats when injected in to the PVT, whereas inhibition of orexin attenuates anxiety [30,43,45,53]. HCN4 channels are highly expressed in the basolateral amygdala (BLA) [38], and HCN channel blockade in the BLA causes anxiety [44]. HCN channels also play important roles in the functional modulation of the midbrain dopamine (DA) system [12,37,41] which

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Table 1
Single marker association analysis.

SNP/position	*N*	Minor	%	*P*	Bonf.	*Q*-Value	OR/95% CI		Genotypes		PHWE
Block 1								C/C	C/T	T/T	
rs498005/73620310	Case (259)	C	49.0	**0.033**	0.297	0.069	1.34/	21.2	55.6	23.2	0.08
	Control (337)		43.9				1.02–1.76	20.2	47.5	32.3	0.51
								A/A	A/G	G/G	
rs3859014/73626439	Case (251)	A	35.3	**0.047**	0.423	0.069	0.74/	7.6	55.4	37	0.00
	Control (335)		39.6				0.54–0.99	14.3	50.4	35.2	0.36
								G/G	G/T	T/T	
rs546564/73627770	Case (265)	G	39.8	0.477	1.000	0.236	1.11/	13.2	53.2	33.6	0.09
	Control (341)		38.4				0.84–1.45	14.7	47.5	37.8	1.00
Block 2								C/C	C/G	G/G	
rs548525/73627871	Case (266)	C	13.9	0.081	0.729	0.072	1.43/	1.1	25.6	73.3	0.44
	Control (341)		12.0				0.96–2.13	0.9	22.3	76.8	0.45
								T/T	T/C	C/C	
rs12905211/73628168	Case (265)	T	51.5	**0.004**	**0.036**	**0.018**	1.5/	23.4	56.2	20.4	0.05
	Control (345)		42.2				1.13–1.98	17.1	50.1	32.7	0.66
								C/C	C/A	A/A	
rs8030574/73628214	Case (281)	C	26.3	0.076	0.684	0.072	1.31/	6.0	40.6	53.4	0.54
	Control (345)		23.2				0.97–1.77	5.5	35.4	59.1	0.88
Block 3								A/A	A/G	G/G	
rs2623997/73628714	Case (266)	A	50.8	0.138	1.000	0.102	1.23/	23.3	54.9	21.8	0.14
	Control (339)		45.4				0.94–1.61	20.9	49	30.1	0.83
								A/A	A/G	G/G	
rs4776632/73632376	Case (260)	A	43.3	0.429	1.000	0.236	0.9/	17.3	51.9	30.8	0.38
	Control (331)		48.8				0.68–1.18	23.6	50.4	26.0	0.91
								A/A	A/T	T/T	
rs3784812/73659194	Case (265)	A	9.1	0.300	1.000	0.190	1.29/	1.1	15.8	83	0.46
	Control (306)		7.7				0.8–2.07	0.6	14	85.3	0.69

Abbreviations: *P*, *P*-value for SNP association; Bonf., Bonferroni corrected *P* value; *Q*-value, false-discovery rate corrected *P* value; OR, odds ratio; CI, confidence interval; PHEW, *P*-values for Hardy-Weinberg equilibrium. All association analyses were adjusted for the effects of age and sex.

has been implicated in depression and other mood disorders [39].

Because HCN4 channels may regulate mood and anxiety by affecting the function of the thalamus, amygdala, and midbrain DA systems, and may indirectly influence PFC function, *HCN4* is a good candidate gene for mood and anxiety disorder risk. We therefore tested for association of *HCN4* genotype with a group of mood and anxiety disorders – MDD, bipolar disorder, and OCD. The positive association findings described here are consistent with a role for HCN4 in mood and anxiety disorders and motivate future research into the role of HCN channels in these disorders.

Variation in *HCN4* on chromosome 15 was characterized in 285 Caucasian patients (mean age = 43.4 ± 11.9 years and 35% male) and 354 Caucasian controls (mean age 61.0 ± 17.9 years and 43% male). The patients included in this study met DSM-IV criteria for mood and/or anxiety disorders as assessed using the Structured Clinical Interview for DSM Disorders (SCID-RV), and included 43 patients with bipolar disorder, 84 with obsessive-compulsive disorder, and 174 with major depressive disorder. Among the bipolar subjects, 11 had a co-morbid anxiety disorder, and among the major depressive disorder patients 20 had a co-morbid anxiety disorder. A total of 13 of the obsessive compulsive disorder cases had co-morbid major depressive disorder. The case phenotype was scored as *Present* if a subject was found to have major depression, bipolar I, bipolar II, and/or obsessive-compulsive disorder. Both healthy controls and patients were recruited via radio advertisement, study flyers and the internet. Patients and controls were assessed using the SCID-RV. Controls had no current or past DSM-IV diagnoses apart from possible nicotine abuse. A standard informed consent was obtained from all subjects. This work was approved by the Yale University Human Investigation Committee.

We selected nine tag SNPs in *HCN4* using Haploview software (www.broad.mit.edu/mpg/haploview/) with the Tag SNP Picker routine and Hapmap data to cover all 38.9 kb of the *HCN4* gene. These SNPs met the criteria of being in Hardy-Weinberg equilibrium in the HapMap sample (*P* value ≥ 0.05), an r^2 threshold ≥ 0.8 and minimum allele frequency of ≥7.7% based on Hapmap data (http://hapmap.ncbi.nlm.nih.gov/). Additional SNPs were not included to minimize multiple testing. SNP genotypes were obtained using Sequenom iPLEX Gold on a Sequenom MassARRAY system maintained by the Yale Keck Center. All primer sequences are available upon request.

Analyses were conducted using the *SNPassoc*, *genetics*, and *haplo.stats* packages in 'R' (cran.r-project.org). The reported *P* values correspond to log-additive models. All analyses included age and sex as covariates. For the analysis of the linkage disequilibrium (LD) pattern and haplotype block delineation we used Haploview. We corrected *P* values using Bonferroni correction for multiple testing as well as using the *Q*-value package in R (http://cran.r-project.org/web/packages/qvalue/index.html). We also calculated sample sizes (samples per group) required for power = 0.8 with alpha = 0.05 based on the observed effect sizes by simulation in R for the non-significant single marker analyses (rs546564 (*n* = 9083), rs548525 (*n* = 2344), rs8030574 (*n* = 1451), rs2623997 (*n* = 662), rs4776632 (*n* = 619), and rs3784812 (*n* = 2966)).

All SNPs were in Hardy-Weinberg equilibrium (HWE) in controls. In patients, SNP rs3859014 was not in HWE and SNP rs12905211 had a *P* value of borderline significance (see Table 1), suggesting the possibility that these variants influence disease risk [26]. We found evidence for nominal association between three SNPs (rs498005, rs3859014 and rs12905211) and this group of mood and anxiety disorders (*P* = 0.033, 0.047 and 0.004, respectively). SNP rs12905211 maintained significance after Bonferroni correction (*P* = 0.035) with the T allele being more frequent (OR = 1.5; 95% CI = 1.13–1.98; see Table 1) in cases compared to controls. SNPs rs498005 and rs3859014 did not maintain significance after Bonferroni correction (*P* = 0.297 and *P* = 0.423, respectively). Putative LD blocks were identified. Block 2, including SNPs rs548525, rs12905211 and rs8030574, had two significant associated haplotypes, haplotype CTC, with *P* = 0.004 and GTA, *P* = 0.02, but only the former was significant after Bonferroni correction (OR = 2.88; 95% CI = 1.41–5.90), and haplotype CGT in Block 1 approached significance (see Table 2).