Sequence polymorphisms of *MC1R* gene and their association with depression and antidepressant response

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Objective Melanocortin 1 receptor (MC1R) is involved in various functions, such as pigmentation, antipyretic and anti-inflammatory actions, development of melanoma, susceptibility to ultraviolet-induced sun damage, modification of oculocutaneous albinism, development of freckles, and mediation of female-specific mechanisms of analgesia. MC1R's natural agonists include α -melanocyte-stimulating hormone and corticotrophin (ACTH1-39), which are important components of hypothalamic pituitary adrenal axis and increase in response to stress. Given the multiple relevant roles of MC1R, we studied whether the *MC1R* gene would be associated with susceptibility to major depressive disorder or with response to antidepressant treatment.

Methods The human *MC1R* gene is highly polymorphic; therefore, we sequenced the entire *MC1R* coding region of 1122 bp in 181 depressed Mexican-American patients and 185 Mexican-American controls.

Results A total of 23 single nucleotide polymorphisms (SNPs, 15 known and eight new) were found within the sequenced region. Among the common SNPs, the nonsynonymous SNP, rs885479 (R163Q) was associated with the diagnosis of depression ($P=0.04$). The nonsynonymous SNP, rs2228479 (V92M) and the synonymous SNP, rs2228478 were found to be associated with the remission with desipramine treatment. No

associations were found for remission with fluoxetine treatment or for the combined sample treated with fluoxetine or desipramine. The frequency of one (H2) of the five haplotypes identified was higher in depressed patients when compared with controls ($P=0.05$). In-silico functional analysis indicates that SNPs rs885479 and rs2228479 have significant impact on the protein function.

Conclusion The *MC1R* gene might be associated with major depressive disorder and with treatment response to desipramine. *Psychiatr Genet* 21:14–18 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Major depressive disorder (MDD) is a common and complex gene–environment disorder clinically characterized by pervasive low mood, loss of interest in usual activities, diminished ability to experience pleasure (anhedonia), and fatigue. Other key central nervous system functions such as sleep, appetite, temperature and neuroendocrine regulation, locomotion, and cognition may also be altered. The onset of a depressive episode requires a genetic background of increased susceptibility combined with the presence of stressful life events. Consequently, it has been assumed that genes that exacerbate or buffer the effect of life stressors may contribute to MDD risks. A functional polymorphism in the promoter region of the serotonin transporter (5-HTT) gene has been shown to moderate the influence of stressful life events in depression (Caspi *et al.*, 2003). Given that the selective serotonin reuptake inhibitors are among the most effective antidepressants, this finding

was plausible and regarded as further evidence for the neurotransmitter hypothesis of depression. But follow-up studies failed to replicate this finding completely (Risch *et al.*, 2009). The meta-analysis showed that the stressful life events are a potent risk factor for depression, but the 5-HTT genotype was not shown to be a predisposition factor for developing depression either along or interacting with stressful life events (Risch *et al.*, 2009). Several alternative theories for the mechanism of depression susceptibility have also been proposed, such as the neuroendocrine hypothesis, which studies the role hypothalamic pituitary axis overactivation and inhibition of counterproductive neurovegetative function in chronic life stress. The neurotrophic hypothesis implicates the role of neurotoxic and neuroprotective effects in the depression development, and neurotrophins, such as the brain-derived neurotrophic factor are postulated to have a key role in the pathophysiology of depression and antidepressant action (Groves, 2007). The neuroimmune

hypothesis has been concerned with the interactions between the central nervous system and the immune system. Recently, a possible immune system dysregulation with Th1 activation in depression has been proposed by Wong *et al.* (Wong *et al.*, 2008). Variants of proteasome subunit- β type 4 and *T-box-21* genes were associated with MDD and antidepressant treatment (Wong *et al.*, 2008). As a complex trait with both genetic and nongenetic contributory factors, potential new genetic factors associated with susceptibility of MDD are still under vigorous investigation using genome-wide association study strategies. Well-designed pharmacogenomic studies require prospective double-blind design that is not particularly suitable for genome-wide association studies because of the limitation of the number of patients who can be enrolled prospectively. For that reason it is reasonable to propose candidate gene studies of small, but very well characterized pharmacogenomic samples.

Signal transduction mediated by proopiomelanocortin protein (POMC) represents a logical candidate pathway for pharmacogenomic studies of depression. POMC consists of 241 amino acids, which could be classified as having three main domains: the central highly conserved corticotrophin (ACTH1–39) sequence, with α -melanocyte-stimulating hormone (MSH) at its *N*-terminus; the *C*-terminal β -lipotropin, which can be cleaved to generate β -endorphin; and the *N*-terminus region that contains γ -MSH (Takahashi *et al.*, 1981; Castro and Morrison, 1997). Posttranslational processing of the POMC protein ultimately leads to the generation of various hormones with distinct bioactivities, such as the melanocortins (α -, β -, and γ -), β -lipotropin, γ -lipotropin, β -endorphin, and ACTH. Melanocortins exert their biological effects by binding to the smallest family of stimulate G-protein-coupled transmembrane receptors, the melanocortin receptors (MCR). Five MCRs have been identified and termed MC1R to MC5R, with various homologous between each other. MC1R is a 317 amino acid protein, expressed in various tissues including melanocytes, macrophage cells, human monocytes, neutrophils, endothelial cells, fibroblasts, mast cells and lymphocytes, and in rat and human brains in neurons of the periaqueductal gray substance (Star *et al.*, 1995; Xia *et al.*, 1995; Catania *et al.*, 1996; Bhardwaj *et al.*, 1997; Hartmeyer *et al.*, 1997; Adachi *et al.*, 1999; Taherzadeh *et al.*, 1999; Andersen *et al.*, 2001). Besides, α -MSH, and ACTH1–39 also can serve as active peptide for MC1R (Abdel-Malek, 2001). Until now, many different functions have been attributed to this receptor, including hair and skin pigmentation, antipyretic and anti-inflammatory actions, development of melanoma, susceptibility to ultraviolet-induced sun damage, modification of oculocutaneous albinism, and development of freckles and solar lentigines (Jackson, 1993; Valverde *et al.*, 1995, 1996; Bastiaens *et al.*, 2001; King *et al.*, 2003; Rees, 2004; Nakayama *et al.*, 2006). MC1R also mediates female-specific mechanisms of analgesia through κ -opioid receptor in mice and humans (Mogil *et al.*, 2003; Getting,

2006). Given the multiple function of MC1R, here we explored whether polymorphisms of the *MC1R* gene are associated with MDD and antidepressant response in Mexican-Americans.

Methods

Participants

DNA samples from 280 Mexican-Americans (95 depressed patients and 185 age-matched and sex-matched healthy controls) were obtained from earlier studies (Licinio *et al.*, 2004; Wong *et al.*, 2006), whereas 86 depressed Mexican-American samples were newly recruited according to the same criteria as described earlier (Wong *et al.*, 2006). All the participants had at least three biological grandparents of Mexican heritage. Among the depressed participants, 95 individuals were enrolled in a pharmacogenetic study of antidepressant treatment response to desipramine or fluoxetine. This study was approved by the Institutional Review Boards of the University of California Los Angeles, University of Miami, and John Curtin School of Medical Research, and has been registered in ClinicalTrials.gov (NCT00265292).

Genotyping

Sequencing was used for screening SNPs on the *MC1R* gene for the existing 366 DNA samples. The entire coding region of the *MC1R* gene was amplified using PCR primers, MC1R_F (5'-GGC AGC ACC ATG AAC TAA GC-3') and MC1R_R (5'-GGT CAC ACA GGA ACC AGA CC-3') to yield a 1122-nucleotide product containing 5' and 3' flanking sequences. In brief, amplification was performed in 20- μ l reaction volumes, containing 100-ng DNA, 0.2 μ mol/l of each primer, 0.2 mmol/l of each dNTP, 1X PCR buffer, and 1 U of AmpliTaq (Applied Biosystems Inc., Foster City, California, USA). PCR was initiated at 95°C for 2 min and performed for 35 cycle, each consisting of 1 min at 94°C, 1 min at 65°C, and 1 min at 72°C. The PCR products were cleaned up using ExoSAP-IT enzyme (USB Corporation, Cleveland, Ohio, USA). Then, the cleaned PCR products were directly sequenced using ABI BigDye v3.1 sequencing Kit on the ABI 3730xl DNA Analyzer (Applied Biosystems) following the manufacturer's protocols. All the PCR products were sequenced for both strands. The primers used for sequencing include the two PCR primers, MC1R_F and MC1R_R, and two inner primers, MC1R_F2 (5'-CAT CTC CAT CTT CTA CGC ACT G-3') and MC1R_R2 (5'-CGT GCT GAA GAC GAC ACT G-3').

Statistical analysis

Maximum likelihood estimates of haplotype frequencies were calculated in control, depressed, remitter, and nonremitter groups using the program Haploview (v4.1; <http://www.broadinstitute.org/haploview>) (Barrett *et al.*, 2005). The statistical significance of the genotype/allele/haplotype frequency variables between the depressed patient and control group and the antidepressant drug response was evaluated by χ^2 test with Yates correction for small numbers.

In-silico functional analysis

The functional impact of the coding SNPs on the protein was estimated by PANTHER software (<http://www.pantherdb.org/tools/csnpscoreForm.jsp>), which calculates the substitution position-specific evolutionary conservation (subPSEC) score based on an alignment of evolutionarily related proteins with known functional information (Brunham *et al.*, 2005; Thomas *et al.*, 2006). The probability of a given variant that will cause a deleterious effect on protein function, $P_{\text{deleterious}}$, was estimated as a function of the subPSEC score. The PANTHER subPSEC scores are continuous values ranged from 0 (neutral) to approximately -10 (most likely to be deleterious). The subPSEC score of -3, which corresponds to a $P_{\text{deleterious}}$ of 0.5, is the cut-off point for functional significance (Brunham *et al.*, 2005).

Results

We sequenced 1122 bp of the entire *MC1R* coding region in 181 depressed Mexican-American patients and 185 healthy controls. In total, 23 SNPs (15 known SNPs in the National Center for Biotechnology Information dbSNP database and eight new SNPs) have been found within the region sequenced (Table 1). The frequency of individuals with at least one SNP is 76.3% ($n = 138$) and 73.5% ($n = 136$) in depressive and control group, respectively. Fifteen nonsynonymous SNPs result in protein amino acid variations, and seven SNPs have the minor frequency higher than 1%. The minor allele frequency of four common SNPs, rs1805005, rs2228479,

rs885479, and rs2228478, is 5.7, 5.9, 30.8, and 11.6%, respectively in the control group (Tables 1 and 2). Among them, the SNP rs885479, with the highest minor allele frequency in the study population, has been found to be associated with depression ($P = 0.04$).

We also found that two SNPs, rs2228479 and rs2228478, were associated with desipramine response (Table 2). The frequency of allele A at SNP rs2228479 was 12% in the remitter group, which was significantly higher than that in the nonremitter group (0%) when treated with desipramine. Similarly, the frequency of G allele at SNP rs2228478 was 20% in the remitter group, which was significantly higher than that in the nonremitter group (2.9%) treated with desipramine. No association was found for remission with fluoxetine treatment, or considering the combined sample of patients treated with both fluoxetine and desipramine.

Five major haplotypes have been found in the study population, which make up 93.1% of the study population (Table 3). We defined the haplotypes as H1 to H5 based on their frequency from high to low. Their frequencies are 49.4, 31.8, 4.9, 4.6, and 2.4%, respectively. Among the five haplotypes, the frequency of H2 is 33.2% in the depressed group, and 28.4% in the control group. The statistical test for the frequency difference of H2 is on the critical significant point, $P = 0.05$.

In-silico functional analysis of the nonsynonymous SNPs associated with depression and desipramine response was carried out. The subPSEC scores ($P_{\text{deleterious}}$) of SNPs,

Table 1 *MC1R* genotype and allele frequencies in Mexican-Americans

Order	SNP	Chromosome position	Allele	Phenotype	Depressed patient			Control		
					Genotype (N=181)			Genotype (N=185)		
					Major/major	Major/minor	Minor/minor	Major/major	Major/minor	Minor/minor
1	New	88513294	G→A	G43R	181 (100)	0 (0)	0 (0)	184 (99.5)	1 (0.5)	0 (0)
2	rs1805005	88513345	G→T	V60L	163 (90.1)	18 (9.9)	0 (0)	165 (89.2)	19 (10.3)	1 (0.5)
3	New	88513416	G→A	S83S	181 (100)	0 (0)	0 (0)	184 (99.5)	1 (0.5)	0 (0)
4	rs1805006	88513419	C→A	D84E	180 (99.4)	1 (0.6)	0 (0)	184 (99.5)	1 (0.5)	0 (0)
5	rs2228479	88513441	G→A	V92M	167 (92.3)	13 (7.2)	1 (0.6)	164 (88.6)	20 (10.8)	1 (0.5)
6	rs34158934	88513451	C→T	T95M	180 (99.4)	1 (0.6)	0 (0)	185 (100)	0 (0)	0 (0)
7	New	88513476	C→T	A103A	181 (100)	0 (0)	0 (0)	184 (99.5)	1 (0.5)	0 (0)
8	rs3212364	88513485	G→A	L106L	180 (99.4)	1 (0.6)	0 (0)	183 (98.9)	2 (1.1)	0 (0)
9	New	88513500	G→A	A111A	177 (97.8)	4 (2.2)	0 (0)	182 (98.4)	3 (1.6)	0 (0)
10	rs11547464	88513592	G→A	R142H	179 (98.9)	2 (1.1)	0 (0)	183 (98.9)	2 (1.1)	0 (0)
11	rs1805007	88513618	C→T	R151C	181 (100)	0 (0)	0 (0)	183 (98.9)	2 (1.1)	0 (0)
12	rs1110400	88513631	T→C	I155T	180 (99.4)	1 (0.6)	0 (0)	182 (98.4)	3 (1.6)	0 (0)
13	rs1805008	88513645	C→T	R160W	181 (100)	0 (0)	0 (0)	184 (99.5)	1 (0.5)	0 (0)
14	rs885479^a	88513655	G→A	R163Q	79 (43.6)	69 (38.1)	33 (18.2)	91 (49.2)	74 (40)	20 (10.8)
15	rs3212366	88513753	T→C	F196L	180 (99.4)	1 (0.6)	0 (0)	185 (100)	0 (0)	0 (0)
16	New	88513866	G→A	Q233Q	180 (99.4)	1 (0.6)	0 (0)	184 (99.5)	1 (0.5)	0 (0)
17	New	88513910	G→A	Q248D	181 (100)	0 (0)	0 (0)	184 (99.5)	1 (0.5)	0 (0)
18	New	88513970	C→G	P268R	181 (100)	0 (0)	0 (0)	184 (99.5)	1 (0.5)	0 (0)
19	New	88513987	G→A	G274S	181 (100)	0 (0)	0 (0)	184 (99.5)	1 (0.5)	0 (0)
20	rs1805009	88514047	G→C	R294P	177 (97.8)	3 (1.7)	1 (0.6)	183 (98.9)	2 (1.1)	0 (0)
21	rs3212367	88514067	C→T	F300F	181 (100)	0 (0)	0 (0)	184 (99.5)	1 (0.5)	0 (0)
22	rs2228478	88514109	A→G	T314T	155 (85.6)	25 (13.8)	1 (0.6)	146 (78.9)	35 (18.9)	4 (2.2)
23	rs3212368	88514133	G→A	3'UTR	177 (97.8)	4 (2.2)	0 (0)	180 (97.3)	5 (2.7)	0 (0)

MC1R, melanocortin-1 receptor; SNP, single nucleotide polymorphism.

^aThis SNP, shown in bold, is significantly associated with depression ($P=0.04$).

Table 2 Four common *MC1R* mutations and status of remission

SNP	Test	NR vs. R, desipramine + fluoxetine					NR vs. R, desipramine					NR vs. R, fluoxetine				
		NR	R	χ^2	d.f.	P	NR	R	χ^2	d.f.	P	NR	R	χ^2	d.f.	P
rs1805005 (V60L)	ALLELIC	5/61	6/102	0.28	1	0.60	2/32	3/47	0.00	1	0.98	3/29	3/55	0.59	1	0.44
rs2228479 (V92M)	ALLELIC	1/65	7/101	2.3	1	0.13	0/34	6/44	4.39	1	0.04 ^a	1/31	1/57	0.19	1	0.67
rs885479 (R163Q)	ALLELIC	28/38	37/71	1.17	1	0.28	16/18	14/36	3.2	1	0.07	12/20	23/35	0.04	1	0.84
rs2228478 (T314T)	ALLELIC	3/63	13/95	2.75	1	0.10	1/33	10/40	5.18	1	0.02 ^a	2/30	3/55	0.05	1	0.83

D.f., degree of freedom; *MC1R*, melanocortin-1 receptor; NR, nonremitter; R, remitter; SNP, single nucleotide polymorphism.

^aThe SNPs are associated with desipramine response ($P < 0.05$).

Table 3 *MC1R* haplotypes from 23 locus (SNP1–23)^a and depression status in Mexican-Americans

Haplotype	Sequence ^b	Frequency	Case-control ratios	χ^2	P value
H1	GGGCGCCGGGCTCGTGGCGGCAG	0.494	174.1 : 187.9, 187.4 : 182.6	0.477	0.4898
H2	GGGCGCCGGGCTCATGGCGGCAG	0.318	127.3 : 237.4, 105.3 : 264.7	3.397	0.0500
H3	GTGCGCCGGGCTCGTGGCGGCAG	0.049	16.5 : 345.5, 19.2 : 350.8	0.152	0.6965
H4	GGGCAACGGGCTCGTGGCGGCAG	0.046	14.0 : 348.0, 20.0 : 350.0	0.978	0.3227
H5	GGGCGCCGGGCTCGTGGCGGCAG	0.024	6.3 : 355.7, 11.4 : 358.6	1.365	0.2400

MC1R, melanocortin-1 receptor; SNP, single nucleotide polymorphism.

^aSee Table 1 for definitions of the SNP1–23, and the haplotype is from 5' to 3' in *MC1R*.

^bThe SNPs shown in bold and underlined in the haplotype are different ones compared with most common haplotype H1.

rs885479 (R163Q) and rs2228479 (V92M) are -4.27401 (0.78143) and -4.54511 (0.82421), respectively. The subPSEC scores indicate that both SNPs have significant impact on the protein function.

Discussion

The frequencies of the A allele at SNP rs885479 are 37.3 and 30.8%, respectively in the depressed and control group, which are lower than in Asians (63.3% in Chinese and 75.6% in Japanese, respectively) and higher than in Africans (0%) and Caucasians (7.5%) according to the HapMap database (<http://www.hapmap.org/>). The frequency of the T allele at SNP rs1805005 is 5.7% in the studied population, which is lower than that of Caucasians (10.0%) and higher than that of Asians (0%) and Africans (0%; <http://www.hapmap.org/>). Although Mexican-Americans are considered as a recent mixed population from three major ethnic groups: Spaniard, Africans, and Native Americans (Hanis *et al.*, 1991), our data showed that there is no significant evidence of stratification within the studied population (Dong *et al.*, 2009). Therefore, this Mexican-American population is appropriate for a case-control association study. The *MC1R* allele frequencies are consistent with the gene admixture between Mexican-Americans and other ethnic groups.

Here we found that among the four common SNPs, rs1805005, rs2228479, rs885479, and rs2228478 (Tables 1 and 2), the SNP rs885479 (G → A, R163Q) was associated with depression ($P = 0.04$). We also found that two SNPs, rs2228479 (G → A, V92M) and rs2228478 (A → G, T314T), were associated with remission with desipramine treatment (Table 2). The SNP rs2228479 (V92M), together with the changes at codons 84 and 95, are supposed to alter the α -helix structure of the second

transmembrane domain (Valverde *et al.*, 1995), thereby affecting the function of *MC1R* protein. In-silico functional analysis showed that both SNPs associated with depression and desipramine response have significant functional impact on *MC1R* protein. Variants of *MC1R* gene were reported to be associated with fear of dental pain, anxiety regarding dental care, and avoidance of dental care (Binkley *et al.*, 2009). *MC1R* could mediate female-specific mechanisms of analgesia through κ -opioid receptor in mice and humans (Mogil *et al.*, 2003; Getting, 2006). Desipramine, a tricyclic antidepressant, is also used to treat cocaine and alcohol dependence, neuro-pathic pain, and attention deficit disorder (Gawin *et al.*, 1989). Desipramine was shown to mediate analgesia by blocking norepinephrine reuptake in diabetic neuropathy (Canciani *et al.*, 2006). There was no association with remission with fluoxetine treatment, or with treatment response for the combined sample of patients treated with fluoxetine and desipramine. Fluoxetine, which blocks serotonin uptake, has not been shown to be any more effective than placebo pain relief (Max *et al.*, 1992). It is therefore possible that the *MC1R* may be involved in the remission of depression treated by desipramine through the pain pathway. The risk of depression in women was estimated to be twice as much as that in men (Weissman *et al.*, 1996); this might be intrinsically connected with female-specific mechanisms of analgesia mediated by *MC1R*.

The natural *MC1R* agonists melanocortin and ACTH are derived from the same polypeptide POMC. ACTH is an important component of the hypothalamic pituitary adrenal axis and it is synthesized in the anterior lobe of the pituitary gland in response to biological stress, or produced by cells of immune system (T cells, B cells and macrophages) as a part of the stress response (including

corticotropin-releasing hormone). Therefore, there is also the possibility that MC1R may have a role in susceptibility to depression through the hypothalamic pituitary adrenal axis responses either to stress or to immune regulation, or through other unknown pathways.

Here, we report the novel associations of variants of *MC1R* gene with depression and response to the antidepressant desipramine. Possibly because of the relatively small sample size, these associations were modestly significant. Replication in larger samples will be needed to confirm the associations reported here. As these variants were predicted to have functional effects on the MC1R protein, specific experiments evaluating the functional activity of these variants would be required to confirm those predictions.

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