Journal of Affective Disorders 130 (2011) 487–491

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Brief report

Association between Major Mood Disorders and the *hypocretin receptor 1* gene

Innocenzo Rainero [a,*], Luca Ostacoli [b], Elisa Rubino [a], Salvatore Gallone [a], Luigi Rocco Picci [b], Pierpaola Fenoglio [a], Elisa Negro [a], Carlo Rosso [b], Paola De Martino [a], Mario De Marchi [c], Pier Maria Furlan [b], Lorenzo Pinessi [a]

[a] *Neurology II, Department of Neuroscience, University of Turin, Italy*
[b] *Mental Health Department, San Luigi Gonzaga Hospital, University of Turin, Orbassano, Italy*
[c] *Department of Medical Genetic, San Luigi Gonzaga Hospital, University of Turin, Orbassano, Italy*

ARTICLE INFO

Article history:
Received 28 July 2010
Received in revised form 13 October 2010
Accepted 14 October 2010
Available online 10 November 2010

Keywords:
Major Mood Disorders
Bipolar disorder
Unipolar disorder
Hypocretin system
Hypocretin 1 receptor gene

ABSTRACT

Background: Recent studies suggested a role for hypocretins in the neurobiology of Major Mood Disorders (MMD). The purpose of this study was to investigate hypocretin involvement in MMD evaluating whether particular alleles or genotypes of the hypocretin pathway genes (*HCRT*, *HCRTR1* and *HCRTR2*) would modify the occurrence and clinical features of the disease.
Methods: We selected for the study 229 MMD patients and 259 healthy age-, sex- and ethnicity-matched controls. Cases and controls were genotyped for several single-nucleotide polymorphisms (SNPs) of the *HCRT*, *HCRTR1*, and *HCRTR2* genes.
Results: We found that allelic and genotypic frequencies of the rs2271933 G>A polymorphism (Ile408Val) in the *HCRTR1* gene were significantly different between cases and controls ($p = 0.003$ and $p = 0.0004$, respectively). The carriage of the A allele was associated with a significantly increased disease risk (OR:1.60, 95% C.I. 1.22–2.10). In addition, we found a significant association between HCRTR1 haplotypes and the disease (permutation $p < 0.0001$). In the analysis of subgroups we confirmed the association only in patients with unipolar depression.
Limitations: Our sample was relatively small and included only cases and controls recruited from Northern Italy. Analysis of the disease subgroups warrants reexamination with more subjects. Finally, the effects of the rs2271933 G>A polymorphism on the hypocretin-1 receptor function are unknown.
Conclusions: Our study suggests that the HCRTR1 gene or a linked locus may modulate the risk for Major Mood Disorders and supports recent studies suggesting an involvement of hypocretin neurotransmitter system in affective disorders.

1. Introduction

The Major Mood Disorders (MMD), which include bipolar disorder and major depressive disorder, have a total lifetime prevalence of up to 20% (Kessler et al., 2005), and are associated with increased rates of disability, morbidity, and mortality (Reynolds et al., 2008). Genetic factors may have a significant role in determining susceptibility to MMD (Levinson, 2006). However, no consistent molecular genetic finding has emerged yet.

Hypocretin-1 and -2 (also called orexin-A and -B) are neuropeptides processed from a common precursor, prepro-hypocretin (Sakurai et al., 1998; De Lecea et al., 1998). Two G-protein coupled receptor subtypes, Hcrtr1 and Hcrtr2, have been identified (Smart and Jerman, 2002). Hypocretin-

* Corresponding author. Neurology II, Department of Neuroscience, University of Turin, Via Cherasco 15, 10126 TORINO, Italy. Tel.: +39 011 6334763; fax: +39 011 6638510.
E-mail address: irainero@molinette.piemonte.it (I. Rainero).

0165-0327/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jad.2010.10.033

containing cells are located exclusively in the hypothalamus, with widespread projections to the entire neuroaxis (Peyron et al., 1998). In humans, hypocretins exert an influence on a wide range of physiological and behavioral processes that may be of relevance for several neuropsychiatric diseases (Siegel, 2004; Martynska et al., 2005; Borgland and Labouèbe, 2010).

Recently, studies in animals suggested that hypocretins may be involved in the pathogenesis of mood disorders. Wistar–Kyoto rats, an animal model of depression, have a reduced number of hypothalamic cells expressing Hcrt-immunoreactivity (Allard et al., 2007). Neonatal administration of clomipramine induces in rats a significant alteration of hypocretins concentrations in several brain regions (Feng et al., 2008). Finally, intracerebroventricular administration of hypocretin-1 induces an antidepressant-like effect through hippocampal cell proliferation (Ito et al., 2008).

The purpose of our research was to study the hypocretin system's involvement in Major Mood Disorders evaluating whether a particular allele or genotype of the hypocretin pathway genes (*HCRT, HCRTR1* and *HCRTR2*) would modify the occurrence and clinical features of the disease.

2. Methods

2.1. Subjects

The patients for the study were recruited in three psychiatric wards in Piemonte (Italy). The study population comprised patients consecutively admitted for Major Depressive Episodes or Manic/Hypomanic Episodes between April 2006 and April 2007. Patients with severe medical illnesses or cognitive disorders (MMSE<28/30) that could interfere with the clinical assessment were excluded from the study. Inclusion criteria were age over 18 and an agreement to participate to the study with informed consent. The final sample was constituted by 229 patients (70 males and 159 females; mean age ± SD = 54.0 ± 12.3 yrs). A group of 259 healthy subjects (99 men, 160 women, mean age ± SD = 50.4 ± 12.5 yrs) was used as controls. The controls were blood donors, screened in order to exclude both neurologic and psychiatric disorders. Written informed consent was obtained from all participants and the study was approved by the Ethic Committees of the hospitals involved in the study.

2.2. Clinical data

Axis I diagnoses were evaluated with the Structured Clinical Interview (SCID) for DSM-IV (First et al., 1997) As regards to Axis I diagnoses, 8.4% of the patients had unipolar depression-single episode, 48.5% unipolar depression-recurrent, 12.8% bipolar type II disorder, and 30.4% bipolar type I disorder. The following data were also gathered: age, sex, educational level, age at onset of depression or mania/hypomania, number of episodes of each type, average duration of each phase of illness, number of admissions for mood disorders, family history of psychiatric illness.

2.3. Genetic analysis

Genomic DNA was extracted using the QIAamp® Mini Kit (Qiagen S.p.A.). We examined seven bi-allelic polymorphisms (two for *HCRT* and *HCRTR2*, three for *HCRTR1*) of the hypocretin system, selected from SNPs database of NCBI (www.ncbi.nlm.nih.gov/) that have been shown to be polymorphic in Western populations. We analyzed the polymorphisms by mutation-specific restriction enzyme digestion (MSRED). For all SNPs the nucleotide substitution directly altered the cutting site of the restriction enzyme. In *HCRT* gene we analyzed two SNPs: rs4796777 in 3′ UTR and rs9902709 in intron 1. For *HCRTR1* gene we analyzed SNPs rs10914456, rs4949449, and rs2271933 (Ile408Val). For *HCRTR2* gene we genotyped SNPs rs3122156, and rs2653349 (Val308Ile).For *HCRT* gene rs4796777 Eco147I enzyme was used (modifying a nucleotide in a primer C>G in order to create a site of restriction), for rs9902709 FaqI was used. For *HCRTR1* SNPs rs10914456 NlaIII enzyme was used, for rs4949449 Eco 47I, and for rs2271933 BccI. For genotyping *HCRTR2* gene SNPs rs3122156 RsaI was used, while for rs2653349 MboI. We performed PCR reactions in a final volume of 25 μl, with 90 ng of genomic DNA, 0.4 unit of Taq Gold DNA polymerase, 250 nM of each primer, 1.5 mM $MgCl_2$ and 50 mM dNTPs. We performed an initial denaturation at 95 °C 10 min, and 35 cycles 95 °C 30 s, specific temperature for each couple of primers 30 s, 72 °C 40 s, and a final elongation to 72 °C 7 min. PCR products were electrophoresed on a 1.5% agarose TBE 1X gel and stained with ethidium bromide.

2.4. Statistical analysis

We verified the Hardy–Weinberg equilibrium for all tested populations. We performed statistical analyses using Genepop – version 4.0, SigmaStat – version 3.1 and SPSS — version 17. We used χ^2 test to compare allele (AF) and genotype frequency (GF) between cases and controls. Haploview program version 4.1 (www.broad.mit.edu/mpg/haploview/) was used to examine linkage disequilibrium and to construct haplotype block structures. Both χ^2 analysis and haplotype analysis were followed by a confirming permutation test (100.000 times). Genetic Power Calculation (http://statgen.iop.kcl.ac.uk/gpc) was used to calculate the expected power of the association study (Purcell et al., 2003). ANOVA followed by Bonferroni correction for multiple comparisons was used to analyze the clinical characteristics between cases and controls. According to recent guidelines for case-control association studies, we defined the level of statistical significance at $p<0.01$ (Bird et al., 2001), while for all other comparisons the level was $p<0.05$.

3. Results

3.1. Association between MMD and hypocretin genes

The present study had a power of 0.80 to detect a significant association in AF with an alpha error 0.05, assuming a prevalence of affective disorders = 0.13, a high risk allele frequency = 0.3, a genotypic relative risk GA = 1.5, and a genotypic risk relative risk AA = 2. The two SNPs of the *HCRT* gene we examined (rs4796717 and rs8072081) were not polymorphic in our populations.

Table 1 shows the genotypic (GF) and allelic frequencies (AF) of the five remaining polymorphisms examined (*HCRTR1*: