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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.

Keywords

Major Mood Disorders; Bipolar disorder; Unipolar disorder; Hypocretin system; Hypocretin 1 receptor gene

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 and De Lecea et al., 1998). Two G-protein coupled receptor subtypes, Hcrtr1 and Hcrtr2, have been identified (Smart and Jerman, 2002). Hypocretin-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 and 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 MgCl2 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 $\chi 2$ 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 $\chi 2$ 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: rs2271933, rs10914456, and rs4949449; HCRTR2: rs3122156 and rs2653349). We found a significant difference in both allelic and genotypic frequencies of the HCRTR1 rs2271933 non-synonymous (1222 G > A) polymorphism. AF for 1222G was 69.1% in controls and 58.3% in MMD patients, AF for 1222A was 30.9% in controls and 41.7% in MMD patients ($\chi 2 = 11.880$, $p = 0.0004$). A significant difference in GF between cases and controls was found ($\chi 2 = 11.829$, $p = 0.003$). To reduce the probability of errors, given the observation of a significant difference in rs2271933 analysis, a permutation test (100.000 times) was performed, with an overall $\chi 2$ of 12.346 ($p = 0.0022$). The carriage of the A allele was associated with a significantly increased disease risk (OR:1.60, 95% CI, 1.22–2.10). Subsequently, we analyzed the recessive (AA vs. GA + GG) and dominant (AA + GA vs. GG) models for allele A (Table 2). The comparison between GG vs GA + AA showed a difference between cases and controls ($\chi 2 = 7.76$, $p = 0.005$), with an increased risk of MMD in GA + AA carriers compared to GG carriers (OR = 1.70; 95% CI = 1.16 < OR < 2.50), according to a dominant model.

When we divided MMD patients into different subgroups, according to DSM-IV diagnoses, patients with unipolar depression were significantly different from controls (AF: $\chi 2 = 9.94$, $p = 0.002$; GF: $\chi 2 = 10.162$, $p = 0.006$). In the remaining subgroups the difference did not reach the level of statistical significance. No significant difference in both AF and GF of the remaining examined polymorphisms was found. The different genotypes had no significant effect on the examined clinical characteristics of the disease.

3.2. Haplotype Block Structure Analysis of HCRTR1 and HCRTR2 genes

Pairwise analysis showed that HCRTR1 SNPs are in linkage disequilibrium (LD) with each other: LOD score more than 2 and D' more than 0.8 was found for the three nearby SNPs. These results are in accord with another study concerning LD in the HCRTR1 gene (Meerabux et al., 2005). For the HCRTR1 gene, the analysis identified a total of eight different haplotypes. When haplotypes with a frequency more than 2% were analyzed, the CGG, TTA, TTG and CGA haplotypes were significantly different in cases and controls (p value range: 0.001–0.03).

4. Discussion

Our study shows for the first time that the rs2271933 polymorphism of the HCRTR1 gene is significantly associated with Major Mood Disorders. The carriage of the A allele of this polymorphism was associated with an approximately 2-fold increase in disease risk. Patients homozygous for the AA allele, in comparison with GG genotypes, have a 2.5-fold increased risk of developing the disease. Haplotype analysis confirmed this association. When the patients were divided into subgroups (bipolar type I, bipolar type II and unipolar depression) a significant association was found only in patients with unipolar depression. However, the number of patients examined in the subgroups was probably too low to detect a significant difference. Finally, the different HCRTR1 genotypes do not seem to modify the clinical features examined in our study.

This is the first study showing a significant association between the HCRTR1 gene and MMD. Therefore, additional studies in different populations are needed. The neurobiological mechanisms explaining the relationship between hypocretin transmission and mood disorders are, at present, unknown. We found an association between a non-synonymous polymorphism of the HCRTR1 gene and depression. This polymorphism induces the substitution of a valine residue with an isoleucine in position 408 of the receptor, which could engender an altered receptor function. The Ile408Val mutation of the HCRTR1 gene was first reported as a “benign polymorphism” in human narcolepsy (Wieland et al., 2002). In patients with schizophrenia, the HCRTR1 Ile408Val polymorphism showed a significant association with susceptibility to polydipsia (Meerabux et al., 2005 and Fukunaka et al., 2007). However, the functional effect of this substitution is unknown and additional studies evaluating the effects of this polymorphism on receptor function are needed.

Hypocretin-containing neurons project to all monoaminergic nuclei of the brain, including raphe nuclei (5-HT), locus coeruleus (norepinephrine), and the ventral tegmental area (dopamine) (Peyron et al., 1998 and Fukunaka et al., 2007). Therefore, activation of hypocretin-containing neurons leads to modification of monoaminergic neurons. On the other hand, hypocretin neurones are hyperpolarized by serotonin and catecholamines, thus supporting the presence of a functional positive feedback loop between these different systems (Eriksson et al., 2010). Hypocretin knock-out mice show a significant reduction in the rate of dopamine turnover and a compensatory increase of serotoninergic activity (Mori et al., 2010). The strict relationship between monoamine and hypocretins may be of relevance for the pathogenesis of mood disorders.

Hypocretins are implicated in a variety of functions including feeding, drinking, sleep-wake cycle, cardiovascular function, hormone secretion, pain transmission and autonomic functions (Siegel, 2004, Martynska et al., 2005 and Shirasaka et al., 1999). Recently, hypocretins have been shown to play an important role in drug addiction and reward (Harris et al., 2005 and Harris and Aston-Jones, 2006). All the aforementioned functions are significantly impaired in patients with depression. Few studies in humans have examined hypocretin involvement in depression. The 24-hour CSF hypocretin-1 concentrations significantly differ in depressed patients compared to healthy controls (Salomon et al., 2003), while CSF hypocretin-1 concentrations are significantly decreased in suicidal patients with major depression (Brundin et al., 2007). Further studies evaluating the role of hypocretins in affective disorders are needed.

Another explanation for our data is that the associated polymorphism of the HCRTR1 gene is in linkage disequilibrium with other genes which are responsible for this association. The HCRTR1 gene is located on 1p35.2, a region that has been previously associated with major depression (McGuffin et al., 2005). Additional studies are needed to search for susceptibility genes for mood disorders in this chromosomal region.

5. Conclusion

Our study shows for the first time that a gene of hypocretin neurotransmission system may be involved in Major Mood Disorders. Further studies are warranted in order to elucidate the neurobiological mechanisms underlying our findings and to evaluate potential therapeutic perspectives.

Role of the funding source

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Conflict of interest

All authors declared that they have no conflicts of interest.

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