

A polymorphism of the *hypocretin receptor 2* gene is associated with cluster headache

I. Rainero, MD, PhD; S. Gallone, MD; W. Valfrè, MD; M. Ferrero, MD; G. Angilella, MD; C. Rivoiro, MD; E. Rubino, MD; P. De Martino, MD; L. Savi, MD; M. Ferrone, MD; and L. Pinessi, MD

Abstract—Several polymorphisms of the hypocretin/orexin system genes were evaluated in 109 cluster headache patients and 211 controls. The 1246 G>A polymorphism of the gene was significantly different between cases and controls. Homozygosity for the G allele was associated with an increased disease risk (OR: 6.79, 95% CI, 2.25 to 22.99). The data suggest that the *HCRTR2* gene or a linked locus significantly modulates the risk for cluster headache.

NEUROLOGY 2004;63:1286–1288

Cluster headache (CH) is a primary headache disorder characterized by attacks of severe unilateral, retro-orbital pain accompanied by restlessness and cranial autonomic symptoms. The signature feature of the disease is its periodicity and the patients present a striking unique diurnal and seasonal rhythmicity.

The etiology of CH is still unknown but recent studies suggested that genetic factors play an important role in the disease.¹ Several cases of monozygotic twin pairs concordant for CH have been reported in the literature. In some families, the phenotype is inherited as an autosomal dominant trait. Finally, in comparison with the general population, both first- and second-degree relatives of CH patients have a significantly increased risk for the disease. At present, however, the type and the number of the genes involved in CH are still unclear.

Neuroimaging studies have suggested a fundamental role for the posterolateral hypothalamic gray matter in CH.² Hypocretin-1 and -2 (also called orexin-A and -B) are newly discovered neuropeptides processed from a common precursor, preprohypocretin.³ Hypocretin-containing cells are located exclusively in the posterolateral hypothalamus, with widespread projections to the entire neuroaxis. Two known G(q)-coupled receptors, *Hcrtr1* and *Hcrtr2*,

have been identified. The peptides of the hypocretin/orexin system influence a wide range of physiologic and behavioral processes in mammals.^{4,5} Some of these, such as pain transmission, autonomic, and neuroendocrine functions, may be of relevance for the pathogenesis of CH.

We performed an association study in a cohort of Italian CH patients to evaluate whether a particular allele or genotype of hypocretin/orexin pathway genes (*HCRT*, *HCRTR1*, and *HCRTR2*) would modify the occurrence and the clinical features of CH.

Methods. *Patients.* A total of 109 consecutive, unrelated patients with CH (85 men, 24 women; mean age \pm SD = 43.8 \pm 12.1 years) were involved in the study. The diagnosis of CH was made according to the International Classification of Headache Disorders (IHCID-II) criteria.⁶ Ninety-six patients fulfilled the diagnostic criteria for episodic CH and 13 for chronic CH. Age at onset and duration of the disease were 24.3 \pm 11.3 years and 19.0 \pm 12.3 years. Twenty-seven (26.2%) of the patients reported a positive family history for migraine and five (4.9%) for CH. A group of 211 age and geographically (Northern Italy) matched healthy subjects (160 men, 51 women, mean age \pm SD = 43.3 \pm 11.9 years) were used as controls. The controls were blood donors and were screened by a neurologist specialized in headaches in order to exclude CH and migraine. Written informed consent was obtained from all participants and the study was approved by the Hospital Ethics Committee.

Genetic analysis. Genomic DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen SpA, Milan, Italy). We examined six polymorphisms (two for each gene—*HCRT*, *HCRTR1*, and *HCRTR2*) of the hypocretin/orexin system (NCBI single nucleotide polymorphisms [SNPs] database—www.ncbi.nlm.nih.gov). In the promoter region of *HCRT* gene we analyzed two polymorphisms:

Additional material related to this article can be found on the *Neurology* Web site. Go to www.neurology.org and scroll down the Table of Contents for the October 12 issue to find the title link for this article.

From the Neurology III Headache Center, Department of Neuroscience (Drs. Rainero, Valfrè, Ferrero, Rivoiro, Rubino, De Martino, Savi, and Pinessi), and SCU Medical Genetics (Drs. Gallone, Angilella, and Ferrone), University of Turin, Italy.

The study was supported by a 2003 grant from the Ministero dell'Università e della Ricerca Scientifica (MURST) and Regione Piemonte (Italy).

Received June 21, 2004. Accepted in final form August 4, 2004.

Address correspondence and reprint requests to Dr. Innocenzo Rainero, Neurology III Headache Center, Department of Neuroscience, University of Turin, Via Cherasco 15-10126 Turin, Italy; e-mail: irainero@molinetto.piemonte.it

Table 1 Genotype distribution and allele frequencies of the polymorphisms of the HCRTR1 and HCRTR2 genes

	n	Genotype	Genotype	Genotype	AF	
HCRTR1 264 T>C		T/T (%)	T/C (%)	C/C (%)	T	C
CH patients	109	28 (25.7)	66 (60.6)	15 (13.8)	0.56	0.44
Controls	211	71 (33.6)	107 (50.7)	33 (15.6)	0.59	0.41
HCRTR1 1375 C>T		C/C (%)	C/T (%)	T/T (%)	C	T
CH patients	109	56 (51.4)	45 (41.3)	8 (7.3)	0.72	0.28
Controls	211	117 (55.5)	70 (33.2)	24 (11.4)	0.72	0.28
HCRTR2 1246 G>A		G/G (%)	G/A (%)	A/A (%)	G	A
CH patients	109	103 (94.5)	4 (3.7)	2 (1.8)*	0.96	0.04†
Controls	211	163 (77.3)	43 (20.4)	5 (2.4)	0.87	0.13
HCRTR2 IVS4 + 12.564 A>C		C/C (%)	C/A (%)	A/A (%)	C	A
CH patients	109	9 (8.3)	96 (88.1)	4 (3.7)	0.52	0.48
Controls	211	10 (4.7)	191 (90.5)	10 (4.7)	0.50	0.50

Values are the number (%) of patients positive for each genotype. *p* Values were calculated by the χ^2 test from 3×2 (genotype) or 2×2 (allele) contingency tables.

* CH patients in comparison with controls: χ^2 16.3, *p* = 0.0003, power = 0.97.

† CH patients in comparison with controls: χ^2 4.1, *p* = 0.042, power = 0.52.

CH = cluster headache; AF = allele frequencies.

-3250C>T (SNP # 4796717) and -1717C>T (SNP # 8072081). In the *HCRTR1* gene we analyzed the polymorphisms 264T>C (R37R) (SNP # 1056526) in exon 1 and 1375C>T (I408V) (SNP # 2271933) in exon 7. For *HCRTR2* gene, SNP 1246G>A (V308I) (SNP # 2653349) in exon 5 and the IVS4 + 12.564A>C (SNP # 1027650) polymorphisms were genotyped. The SNP IVS4 + 12.564C>A polymorphism was analyzed using an allele-specific oligonucleotide (ASO) hybridization (PCR-ASO). The remaining polymorphisms were analyzed by PCR-ARLS (Allele Restriction Creation Site) (see the supplementary data on the *Neurology* Web site at www.neurology.org).

Statistics. The Hardy-Weinberg equilibrium was verified for all tested populations. Statistical analyses were performed using SigmaStat, version 1.0 (Jandel Corp., 1994, San Rafael, CA). Chi-square test was used to compare allele frequency (AF) and genotype frequency (GF) between cases and controls. The level of significance was taken at *p* < 0.01.⁷

Results. The two SNPs of the *HCRTR* gene that we examined, contrary to previous studies, were not polymorphic in our populations. Table 1 shows the GF and AF of the four remaining polymorphisms examined (*HCRTR1*: 264 C > T and 1375 C > T; *HCRTR2*: 1264 G > A and IVS4 + 12.564 C > A) and the comparison of these frequencies between controls and CH patients. AF for 1246G of the *HCRTR2* gene was 0.87 in controls and 0.96 in CH patients, AF for 1246A was 0.13 in controls and 0.04 in CH patients (χ^2 = 4.11, *p* = 0.042). We found a difference in *HCRTR2* 1246 G>A GF between CH patients and controls (χ^2 = 16.3, *p* = 0.0003, power = 0.97). Subjects homozygous for the G allele have a higher risk for the disease compared to GA genotypes (OR = 6.79; 95% CI = 2.25 < OR < 22.99) (table 2). Homozygosity for the G allele was associated with an increased risk of CH (*p* < 0.0002) compared to GA/AA carriers (OR = 5.06; 95% CI = 1.99 < OR < 13.64) (see table 2). AF and GF frequencies did not differ between males and females (χ^2 = 0.04, *p* = 0.87 in controls and χ^2 = 0.94, *p* = 0.87 in CH patients). GF and AF of the

same polymorphism were similarly distributed between episodic and chronic CH patients. Finally, we divided the patients into different subgroups according to the 1246 G > A polymorphism (GG/GA/AA) of the *HCRTR2* gene. The clinical characteristics of the disease were not significantly different in these subgroups. Genotype and allele frequencies of all the other polymorphisms tested were not significantly different between CH patients and controls.

Discussion. The results of our study show that the 1246 G>A polymorphism of the *HCRTR2* gene is significantly associated with CH. Patients homozygous for the G allele, in comparison with remaining genotypes, have a 5 fold higher risk of developing the disease. The effect was observed in both sexes. When the patients were divided into subgroups (episodic and chronic CH) no significant difference was found. However, the number of chronic CH patients examined is too low in order to detect a statistically significant difference. The different *HCRTR2* genotypes do not seem to significantly modify the main clinical features of the disease.

Genetic association studies are exposed to several biases such as phenotypic definition, adequate sample size of cases and controls and population stratification.⁷ In our study, there are several points reassuring regarding these issues. The diagnosis of CH relies on the IHCD-II criteria that are unambiguous and precise. We increased the statistical power of our study enlarging the number of controls and setting the level of statistical significance at *p* < 0.01. Finally, in our control group, allele and genotype frequencies of the *HCRTR2* 1246 G > A poly-

Table 2 Comparison of genotype distribution and allele frequencies of the 1246 G>A HCRTR2 gene polymorphism between cluster headache patients and healthy controls

	n	Genotype	Genotype	Genotype	AF	
		G/G (%)	G/A (%)	A/A (%)	G	A
Total sample	320	266 (83.1)	47 (14.7)	7 (2.2)	0.90	0.10
Controls	211	163 (77.3)	43 (20.4)	5 (2.4)	0.87	0.13
Male controls	160	123 (76.9)	34 (21.3)	3 (1.9)	0.87	0.13
Female controls	51	40 (78.4)	9 (17.6)	2 (3.9)	0.87	0.13
CH patients	109	103 (94.5)	4 (3.7)	2 (1.8)	0.96	0.04
Male CH patients	85	80 (94.1)	3 (3.5)	2 (2.4)	0.96	0.04
Female CH patients	24	23 (95.8)	1 (4.2)	0 (0.0)	0.98	0.02
	χ^2	<i>p</i>	Power	OR	95% CI	
GG vs GA	14.9	0.0001	0.98	6.79	2.25<OR<22.99	
GG vs AA	0.02	0.88	0.05	1.58	0.27<OR<11.99	
GA vs AA	0.87	0.35	0.14	0.23	0.02<OR<2.41	
AA vs GG+GA	0.01	0.93	0.05	1.30	0.22<OR<9.83	
GG vs GA+AA	14.00	0.0002	0.98	5.06	1.99<OR<13.64	

Values are the number (%) of patients positive for each genotype. *p* values were calculated by the χ^2 test from 3 × 2 (genotype) or 2 × 2 (allele) contingency tables.

CH = cluster headache; AF = allele frequencies.

morphism resulted similar to those previously reported.⁸ However, additional association studies in different populations are necessary in order to confirm *HCRT2* gene involvement in CH.

Our data suggest that the hypocretin/orexin system may be involved in the pathogenesis of CH. Hypocretins have been shown to influence a wide range of physiologic and behavioral processes. Hypocretin neurons play an important role in regulating the sleep-wake cycle and are involved in narcolepsy.⁹ Neuroendocrine functions, stress reactions, sympathetic functions, pain threshold and nociceptive transmission are modulated by these peptides.¹⁰ Several of the above mentioned functions are significantly impaired in patients with cluster CH. So, measurement of the hypocretin concentrations in plasma or CSF of CH patients may be helpful in order to better elucidate the pathophysiological mechanisms of the disease.

An alternative explanation of our data are that the polymorphism of the *HCRT2* gene is in *linkage disequilibrium* with other genes, which are responsible for this association. The *HCRT2* gene is located

on 6p12.1.³ Additional studies are needed to search for susceptibility genes for CH in this chromosomal region.

References

1. Russell MB. Epidemiology and genetics of cluster headache. *Lancet Neurol* 2004;3:279–283.
2. Goadsby PJ. Pathophysiology of cluster headache: a trigeminal autonomic cephalgia. *Lancet Neurol* 2002;1:251–257.
3. Sakurai T, Amemiya A, Ishii M, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 1998;92:573–585.
4. Nishino S. The hypocretin/orexin system in health and disease. *Biol Psychiatry* 2003;54:87–95.
5. Siegel JM. Hypocretin (orexin): role in normal behavior and neuropathology. *Annu Rev Psychol* 2004;55:125–148.
6. Headache Classification Subcommittee of the International Headache Society. The international classification of headache disorders, 2nd ed. *Cephalalgia* 2004;24(Suppl 1):1–151.
7. Bird TD, Jarvik GP, Wood NW. Genetic association studies: genes in search of diseases. *Neurology* 2001;57:1153–1154.
8. Olafsdottir BR, Rye DB, Scammell TE, Matheson JK, Stefansson K, Gulcher JR. Polymorphisms in hypocretin/orexin pathway genes and narcolepsy. *Neurology* 2001;57:1896–1899.
9. Taheri S, Zeitzer JM, Mignot E. The role of hypocretins (orexins) in sleep regulation and narcolepsy. *Annu Rev Neurosci* 2002;25:283–313.
10. Bartsch T, Levy MJ, Knight YE, Goadsby PJ. Differential modulation of nociceptive dural input to [hypocretin] orexin A and B receptor activation in the posterior hypothalamic area. *Pain* 2004;109:367–378.