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Association between the G1246A polymorphism of the *hypocretin receptor 2 gene* and cluster headache: a meta-analysis

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Abstract The objective of this study was to investigate the association between polymorphisms of the *hypocretin receptor 2 gene* (*HCRTR2*) and the risk of cluster headache (CH). The study is a meta-analysis of published case-control studies investigating the association between polymorphisms of the *HCRTR2* gene and CH. Pooled odds ratios (OR) were estimated using both random (RE) and fixed effects (FE) models. Three studies, performed in five different European countries, with 593 cases and 599 controls, were included in the study. Allele G of the G1246A HCRTR2 polymorphism was significantly associated with CH (FE OR 1.58, CI 95% 1.27–1.95; RE OR 1.55 (1.14–2.12)). Carriers of the GG genotype showed a higher disease risk compared to the remaining genotypes (FE OR 1.75, CI 95% 1.37–2.25; RE OR 1.69, CI 95% 1.11–2.58). Our data confirm that the G1246A polymorphism of the *HCRTR2* gene may modulate the genetic risk for CH.

Keywords Meta-analysis • Genetic association • Cluster headache • G1246A polymorphism • *Hypocretin receptor 2 gene*

Introduction

Cluster headache (CH) is one of the most painful types of primary headaches. The disease consists of attacks of sudden, severe, unilateral periorbital pain, accompanied by restlessness and cranial autonomic symptoms, and it is characterised by a strikingly unique circadian and circannual rhythmicity [1]. Epidemiological studies have shown a lifetime disease prevalence rate of approximately 300 cases per 100.000 [2]. A number of recent observations supported a significant genetic component to CH predisposition: compared with the general population, the first-degree relatives of CH patients had a 14–39-fold increase in the disease risk [3–5]. Genetic epidemiological studies, however, suggested that CH is a complex disease, with several genetic factors interacting with the environment [6–8]. At present, however, no molecular genetic clues have been identified for the disease [8].

The hypocretin receptor 2 gene (*HCRTR2*) gene is located on chromosome 6p12.1; it consists of 7 exons and encodes for a G-protein coupled receptor, exclusively expressed in the brain [9]. *HCRTR2* binds both hypocretin-1 (orexin A) and hypocretin-2 (orexin B) neuropeptides. The hypocretins/orexin system's peptides influence a wide range of physiological and behavioural processes in mammals [10, 11]. The involvement of hypocretins in the transmission of pain, and in autonomic and neuroendocrine functions, may be rel-

evant for the pathogenesis of CH [12, 13].

We have recently shown that the G1246A polymorphism of the *HCRTR2* gene is significantly associated with CH [14]. This association was confirmed by a large study performed in Germany, showing that homozygous carriers of the G allele had a twofold increase in risk for the disease [15]. On the contrary, the association was not replicated in a complex dataset of CH patients of Danish, Swedish, and British origin [16].

The aim of the present work was to further evaluate the association between CH and the *HCRTR2* gene. In order to do so, we performed a meta-analysis of all available studies that evaluated the association between polymorphisms of the *HCRTR2* gene and the disease. Meta-analysis, a statistical tool for combining results across studies, is becoming popular as a method for resolving discrepancies across genetic association studies. In particular, it is a promising method to overcome the problem of small sample size and inadequate statistical power of genetic association studies [17].

Methods

Selection criteria of studies

We identified eligible studies by searching Medline for all publications between January 2004 and December 2006. We used the search terms "cluster headache", "association", and "genetic" in combination with "orexin", "hypocretin receptor 2", "G1246A polymorphism", and "*HCRTR2* gene". We included in our meta-analysis all case-control studies with extractable data. All the included studies were published as full-length articles or letters in peer reviewed journals. The diagnosis of CH was made according to ICHD-II criteria [18].

Data extraction

The following data were extracted from each study: first author, journal, year of publication, ethnicity of study population, genotyping method, allelic and genotypic frequencies of polymorphism, diagnosis of subgroups and number of cases and controls. Whereas they were lacking, we also calculated the allelic and genotypic frequencies of the G1246A polymorphism for cases and controls. Data were extracted by a single investigator; they were then examined by two neurologists expert in headaches.

Statistical analysis

We used Review Manager 4.2, a statistical software package for managing and analysing all aspects of a Cochrane Collaboration systematic review. The effect of the association was indicated as odds ratio (OR) with the corresponding 95% confidence interval (CI). We used both fixed effect (FE) and random effect (RE) to calculate the pooled OR. RE evaluates the heterogeneity between the studies, and it incorporates the between-study variability, representing a more conservative statistical approach. The heterogeneity between studies was quantified using the index I^2. This index is independent of the number of studies included in the meta-analysis and takes values between 0 and 100% (I^2<25% indicates absence of heterogeneity; I^2=25–50% moderate heterogeneity; I^2>50% large heterogeneity). We also used funnel plot in order to assess small-study bias and publication bias. We initially compared the frequency of the G allele with the frequency of A allele. Then we compared GG genotypes with AA genotypes. Finally, we analysed the dominant (GG *vs*. GA+AA) and recessive (GG+GA *vs*. AA) models for allele G.

Results

Eligible studies

A total of three published articles reported on the relationship between the G1246A polymorphism (rs2653349) in the *hypocretin receptor 2 gene* and CH. All three of these studies met the inclusion criteria. The study of Baumber et al. [16] was performed using three different populations, collected in the UK, Sweden and Denmark. So, in the meta-analysis, the three groups were considered separately (Table 1). All the studies were conducted in Caucasian populations. The 1246 G/A polymorphism was genotyped using polymerase chain reaction (PCR) followed by enzymatic digestion with gel electrophoresis as previously described. The study of Baumber et al. [16] also investigated the 224.26 A>C polymorphism (rs3122169) of the HCRTR2 gene, but this polymorphism was not taken into consideration in the present analysis.

Meta-analysis

The study included 593 CH patients and 599 controls, genotyped for the G1246A polymorphism. In all the examined populations, the allele G was the most frequent for the tested polymorphism. In all studies, the distribution of the genotypes in the control groups was in Hardy-Weinberg equilibrium (p<0.05). Graphically, we did not find evidence of publication bias from the funnel plot.

The main analysis investigating the association of the G1246A allele A and the risk of developing CH relative to the allele G showed moderate heterogeneity (p=0.11, I^2=46.2%) between the studies. The pooled ORs showed a significant association with the disease: FE OR: 1.58 (CI 95%

1.27–1.95) (Fig. 1), RE OR: 1.55 (CI 95% 1.14–2.12). The dominant model for allele G (GG vs. GA+AA) produced the same pattern of genotypic association: FE OR: 1.75 (CI 95% 1.37–2.25) (Fig. 2), RE OR: 1.69 (CI 95% 1.11–2.58). There was no significant statistical difference in any other comparison (Table 2).

Discussion

The results of this meta-analysis suggest that genetic variations of the *HCRTR2* gene may influence the risk of CH. Subjects carrying the G allele or the GG genotype of the studied polymorphism present a significantly higher risk (OR 1.58–1.75) of developing the disease.

As well as for all meta-analyses, the results of this study should be considered cautiously. First, the analysis was performed on a relatively small number of retrospective case-control studies and we cannot exclude the possibility of undetected bias. In addition, all the studies were performed in Caucasian populations. So, an ethnicity effect may be involved in this association. Finally, we found a significant heterogeneity between studies and the presence of population stratification cannot be excluded. So additional studies in different populations are necessary to confirm these results.

So far, the possible mechanisms underlying the association between CH and the HCRTR2 gene are unknown. Neurons

Table 1 Distribution of *HCRTR2* G1246A polymorphism in CH patients and controls

	Distribution of G1246A HCRTR2 polymorphism						Frequency of G1246A HCRTR2 alleles			
	GG		GA		AA		G		A	
	CH patients, n (%)	Controls, n (%)	CH patients, n (%)	Controls, n (%)	CH patients, n (%)	Controls, n (%)	CH patients, n (%)	Controls, n (%)	CH patients, n (%)	Controls, n (%)
Rainero (2004) Italy	103 (94)	163 (77)	4 (4)	43 (20)	2 (2)	5 (3)	210 (96)	369 (87)	8 (4)	53 (13)
Schurks (2006) Germany	173 (77)	166 (62)	46 (20)	93 (35)	7 (3)	7 (3)	392 (87)	425 (80)	60 (13)	07 (20)
Baumber (2006) Denmark	56 (58)	37 (51)	38 (40)	31 (43)	2 (2)	4 (6)	150 (78)	105 (73)	42 (22)	39 (27)
Baumber (2006) Sweden	68 (69)	67 (63)	26 (27)	32 (30)	4 (4)	7 (7)	162 (83)	166 (78)	34 (17)	46 (22)
Baumber (2006) UK	41 (65)	57 (64)	20 (32)	2 7 (30)	2 (3)	5 (6)	102 (81)	141 (79)	24 (19)	37 (21)

The corresponding percentages (%) are shown in parentheses

Table 2 Odds ratio and heterogeneity results for the G1246A polymorphism of the *HCRTR2* gene in patients with CH

	Fixed effects OR (95% CI)	Random effects OR (95% CI)	I^2 (%)	*p* value Q test
Alleles G *vs* A	1.58 (1.27–1.95)	1.55 (1.14–2.12)	46.2	0.11
Homozygotes GG *vs* AA	1.57 (0.84–2.92)	1.56 (0.83–2.92)	0	0.88
Dominant model GG *vs* GA+AA	1.75 (1.37–2.25)	1.69 (1.11–2.58)	59.2	0.04
Recessive model GG+GA *vs* AA	1.39 (0.75–2.57)	1.38 (0.74–2.57)	0	0.80

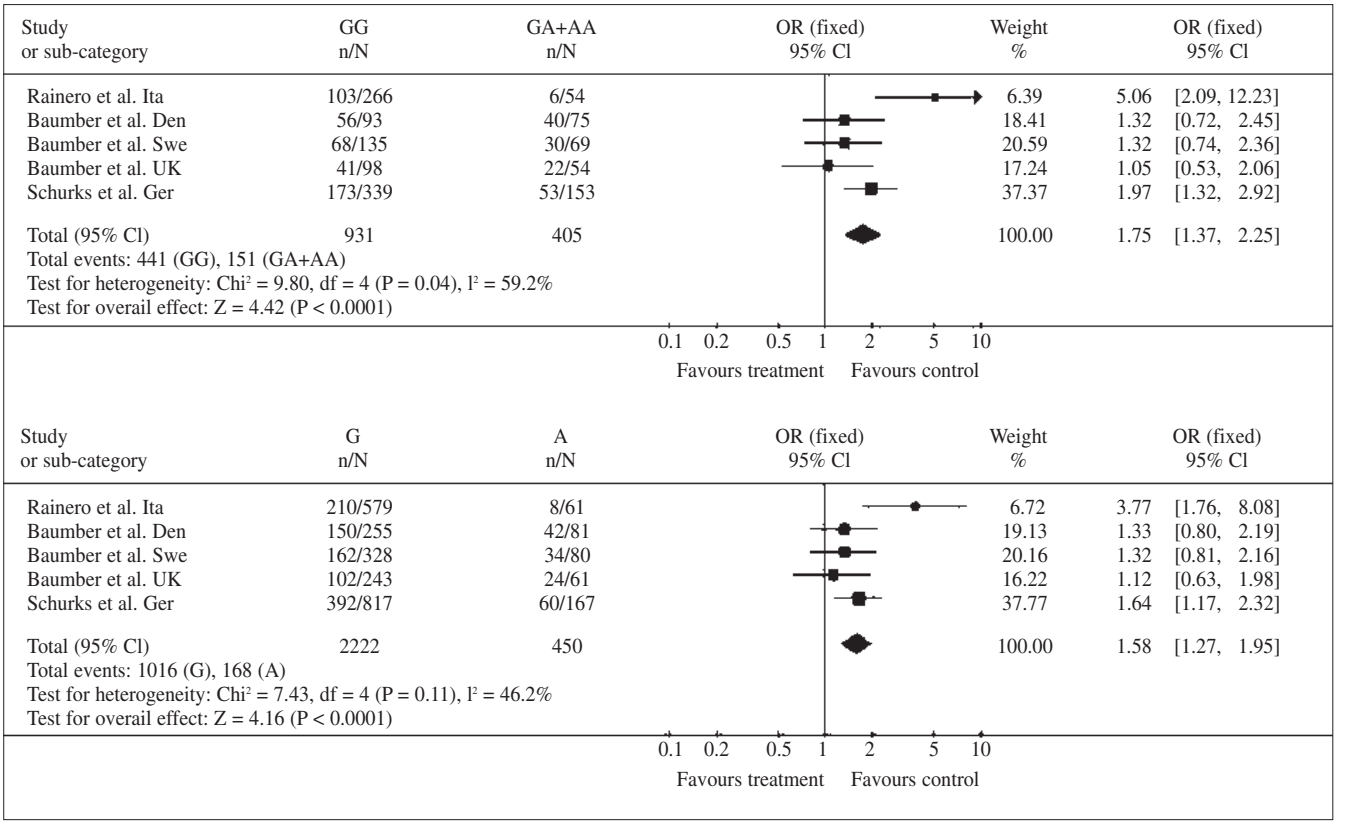

Fig. 1 Meta-analysis for the G1246A polymorphism of the HCRTR2 gene and CH in the comparison between G/G homozygotes *vs*. remaining genotypes (G/A+AA) (top) and between carriers of the G and the A allele (bottom). Values are under both fixed (a) and random (b) effects model. *N* indicates the total number of participants, *n* indicates the number of events

producing hypocretins/orexins are exclusively located in the lateral hypothalamic area, but project broadly to various parts of the brain [19]. Hypocretinergic receptors are widely expressed in the central nervous system, the highest concentrations being found in the neocortex, in the hippocampal formation and in several nuclei of the thalamus, as well as in many hypothalamic and brainstem structures [20, 21]. Neurons containing hypocretin project to multiple neuronal systems, including the noradrenergic ascending nucleus, the serotonergic system of the brainstem, the histaminergic system of the hypothalamus and the cholinergic system in the cerebral cortex [22, 23]. The binding of hypocretins to HCRTR2 receptors promotes calcium influx within the neurons and exerts a post-synaptic excitatory effect on several classes of neurons [24, 25]. The G1246A polymorphism of the *HCRTR2* gene is responsible for an aminoacidic substitution (a valine with an isoleucine in position 308) within the receptor sequence. Our study showed that subjects homozygous for this polymorphism present an increased risk of developing CH. So, additional studies are needed in order to elucidate the functional effects of this aminoacidic substitution on the binding of hypocretins to HCRTR2 receptor and its possible relevance to CH pathophysiology.

In conclusion, our study confirms the presence of an association between CH and the *HCRTR2* gene. This gene may be considered an important genetic risk factor for the disease. Furthermore, our data suggest that the hypocretinergic transmission may have a role in the pathophysiology of CH.

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