Cluster headache and the hypocretin receptor 2 reconsidered: a genetic association study and meta-analysis

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Abstract

Background – Cluster headache is a severe neurological disorder with a complex genetic background. A missense single nucleotide polymorphism (rs2653349; p.Ile308Val) in the *HCRTR2* gene that encodes the hypocretin receptor 2 is the only genetic factor that is reported to be associated with cluster headache in different studies. However, as there are conflicting results between studies we re-evaluated its role in cluster headache.

Methods – We performed a genetic association analysis for rs2653349 in our large LUCA (Leiden University Cluster headache Analysis programme) study population. Systematic selection of the literature yielded three additional studies comprising five study populations, which were included in our meta-analysis. Data were extracted according to predefined criteria.

Results – A total of 575 cluster headache patients from our LUCA study and 874 controls were genotyped for *HCRTR2* SNP rs2653349 but no significant association with cluster headache was found (odds ratio 0.91 (95% confidence interval 0.75-1.10), p=0.319). In contrast, the meta-analysis that included in total 1,167 cluster headache cases and 1,618 controls from the six study populations, which were part of four different studies, showed association of the SNP with cluster headache (random effect odds ratio 0.69 (95% confidence interval 0.53-0.90), p=0.006). The association became weaker, as the odds ratio increased to 0.80, when the meta-analysis was repeated without the initial single South European study with the largest effect size.

Conclusions – Although we did not find evidence for association of rs2653349 in our LUCA study, which is the largest investigated study population thus far, our meta-analysis provides genetic evidence for a role of *HCRTR2* in cluster headache. Regardless, we feel that the association should be interpreted with caution as meta-analyses with individual populations that have limited power have diminished validity.

Key Words: Meta-analysis ■ Genetic association ■ *HCRTR2* gene ■ G1246A polymorphism ■ rs2653349

Introduction

CLUSTER HEADACHE is a primary headache disorder of largely unknown aetiology that is characterized by severe, short-lasting headache attacks accompanied by ipsilateral facial autonomic symptoms and/or restlessness occurring up to eight times a day.[1,2] A role for genetic factors in the aetiology of cluster headache was overlooked for a long time, but has been considered in several studies since the early 1990s.[3-8] Four family studies showed that relatives of cluster headache patients have a higher disease risk,[9-12] but estimates of the relative risk varied considerably between studies, ranging from 14 to 45.[9-11,13] As cluster headache is not as rare as previously thought, it is likely that the disease risk in these studies may have been overestimated.[11] In addition, one of the studies with the highest estimated relative risk[9] may have overestimated the occurance of cluster headache by partly using heteroanamnestic information instead of direct interview or questiannaire data from all affected relatives. Recalculation of the relative risk using a cluster headache prevalence of 0.2% showed a relative risk of 5-18 for first-degree relatives, and 1-3 for second-degree relatives,[13] suggesting a considerably smaller but still relevant contribution of genetic factors in cluster headache.[11] Case reports[3-7,14] on monozygotic twins, both affected by cluster headache, provide further support for a role of genetic factors in the disease. Complex segregation analysis suggested that low-penatrant autosomal dominant genetic risk factor may play a role in a small subset of patients.[34] Most likely, cluster headache is a complex disorder caused by both genetic and environmental factors.[13]

Several genetic studies aimed to identify genes involved in cluster headache but were unsuccessful.[15-18] The role of the *CACNA1A* gene was investigated both in a kindred with three affected family members[16] as well as in an association study,[15,16] but results were negative. Single nucleotide polymorphism (SNP) rs1801133 in *MTHFR* showed no association with cluster headache either.[17] A mitochondrial mutation reported to cause MELAS was identified in a single cluster headache patient without a family history of this disorder,[18] but later studies[19,20] failed to replicate the involvement of mitochondrial mutations in cluster headache. Thus far, genetic research in cluster headache has only led to the identification of one replicated possible genetic susceptibility factor: SNP rs2653349 (G1246A) in *HCRTR2* that encodes the hypocretin type 2 receptor[22-24]. This receptor is expressed in the posterior hypothalamus, which is thought to play an important role in cluster headache.[21] The role of the *HCRTR2* SNP was investigated in five small cohorts in three studies[22-24], comprising in total 593 cases and 599 healthy controls. The minor A allele of SNP rs2653349 was associated with a reduced risk for cluster headache (i.e. the A allele being more frequent in controls than in cases). Rainero *et al.* performed a meta-analysis of all five cohorts and reported association of the *HCRTR2* SNP with cluster

headache[25]. However, the accuracy of the estimated effect size differed largely between studies and the meta-analysis is, therefore, difficult to interpret because (i) there were differences in the statistical models used (i.e. other models than the additive model that is thought to underlie genetic susceptibility of most complex disorders[26]) and ii) there was considerable statistical heterogeneity between the studies.

Using our web-based Leiden University Cluster headache Analysis (LUCA) study population,[27] we re-evaluated the possible association of *HCRTR2* SNP rs2653349 with cluster headache in the largest single study thus far and we subsequently performed new meta-analyses combining our results with those of previous studies.

Materials and Methods

Patient recruitment for the LUCA study

For the LUCA study, self-reported cluster headache patients aged 18 years or older were recruited via our Dutch headache research website (www.lumc.nl/hoofdpijn), which was developed for genetic and epidemiological research on primary headache disorders. Individual diagnoses were established using an extended web-based questionnaire according to the International Classification of Headache Disorders, 2nd edition (ICHD-2)[1] and were validated in a subset of patients by a telephone interview.[27] The medical ethics committee of the Leiden University Medical Centre approved our study and all participants provided written informed consent.

Genetic association study in the LUCA population

All participants that met our algorithm criteria[27] for cluster headache and had provided a DNA sample were included. Our control population consisted of anonymous blood donors of whom no specific health information is available. Based on the low prevalence (0.2%) of cluster headache in the general population, we expected a negligible effect of possibly including one or two patients with cluster headache in our control group. Power calculations were performed using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/),[28] under the assumption of an additive model.

Genotyping of SNP rs2653349 (i.e. DNA variant G1246A) was performed using a TaqMan assay for which probes and primers were designed by Applied Biosystems. A standard PCR reaction was carried out using the TaqMan Universal PCR Master Mix reagent and the genotyping was performed on a Lightcycler LC-480 machine combined with LightCycler®480 1.5.0 software, version 1.5.0.39 (Roche Applied Science) to analyse the genotype clusters. Data analysis was performed using PLINK software version 1.07

(http://pngu.mgh.harvard.edu/~purcell/plink/) by calculating odds ratios (ORs) and 95% confidence intervals (95% C.I.) for the association between the *HCRTR2* SNP rs2653349 and cluster headache. We assumed an additive genetic model, and performed uncorrected and corrected analyses with age and sex as covariates. Association analysis was performed with the major allele (G) as reference. A significance level of 0.05 was used.

Meta-analysis: study selection

According to the guidelines for systematic reviews of genetic association studies[29] two researchers (C.M.W. and L.A.W.) individually searched the literature for genetic studies on the role of *HCRTR2* in cluster headache. We searched the MEDLINE, EMBASE, Pubmed, Web of science, CINAHL, PsychINFO, Academic search Premier, ScienceDirect, LWW, Pubmed Central, Goand Science Citation Index databases (to July 6, 2012) using the following search terms: ("Cluster Headache"[Mesh] OR cluster headache OR Cluster Headaches OR Ciliary Neuralgia OR Ciliary Neuralgias OR Neuralgic Migraine OR Neuralgic Migraines OR Histamine Cephalgia OR Histamine Cephalgias OR Horton Syndrome OR Horton's Syndrome OR Hortons Syndrome OR trigeminal autonomic cephalalgias OR trigeminal autonomic cephalalgia) AND (HCRTR2 OR hypocretin receptor-2 OR HCRTR-2 OR HCRTR 2 OR hypocretin receptor 2 OR HCRTR OR hypocretin receptor OR hypocretin receptors OR OX2R OR orexin OR orexin B OR hypocretin 2 OR rs2653349 OR 2653349 OR G1246A OR G 1246 A OR G1246 A OR G 1246A OR G922A OR G 922 A OR G922 A OR G 922A). We considered all articles published in English. References of all primary articles and reviews on cluster headache genetics were manually searched. Studies were eligible for inclusion if they met the following pre-specified criteria:

1. Study design: only cross-sectional, case–control or cohort studies are eligible.
2. Study must include cluster headache patients and healthy controls.
3. Genotype frequencies of the single nucleotide polymorphism (SNP) *HCRTR2* rs2653349 must be listed in the manuscript. Alternatively, sufficient data to calculate the frequencies must be available from the paper or from the authors upon request.
4. The largest study with extractable data will be selected if multiple studies use the same study population.

Meta-analysis: statistical analysis

Meta-analysis of all study populations was performed in R version 2.15 using the metagen function from the meta package (www.r-project.org). We assumed an additive genetic

model, and modelled the OR both as fixed effect, which assumes homogeneity across studies, and as random effect, which incorporates the between-study variability. The random effect model was defined as our primary model because of the high inter-study variability with respect to sample sizes and estimated effect sizes. The between-study heterogeneity was tested using the I^2 statistic, a Cochrane's Q statistic-based measure that describes the amount of variation due to heterogeneity rather than chance on a continuous scale from 0 to 1. I^2 values of approximately 25%, 50% and 75% are indicative for low, moderate and high between-study heterogeneity, respectively.[30] A funnel plot was generated using SPSS Statistics 20.0 (IBM, Armonk, NY, USA) to check for publication bias. All analyses were performed with the major allele (G) as reference, and a significance level of 0.05 was used.

Table 1 - Baseline characteristics of LUCA cluster headache patient population

	LUCA population N=575
Sex (male, %)	404 (70.3)
Age (year [SD])	49.6 (11.8)
Cluster headache type (episodic)	421 (73.2)
First-degree family member cluster headache (%)	133 (23.2)
Second-degree family member cluster headache (%)	27 (4.7)

Table 2 - Study populations included in the meta-analysis.

			Genotype frequencies			**Allele frequencies**		**Hardy-Weinberg equilibrium**	
Study	**Group**	**N**	**GG**	**GA**	**AA**	**G**	**A**	**χ^2-test**	**p-value**
This study - The Netherlands	Cases	575	351 (61.0%)	206 (35.9%)	18 (3.1%)	908 (79.0%)	242 (21.0%)	3.55	0.059
	Controls	874	522 (59.7%)	307 (35.1%)	45 (5.2%)	1351 (77.3%)	397 (22.7%)	$2.5 \cdot 10^{-4}$	0.987
Rainero et al. - Italy	Cases	109	103 (94.5%)	4 (3.7%)	2 (1.8%)	210 (96.3%)	8 (3.7%)	25.21	$1 \cdot 10^{-6}$
	Controls	211	163 (77.3%)	43 (20.4%)	5 (2.4%)	369 (87.4%)	53 (12.6%)	1.10	0.295
Schurks et al. - Germany	Cases	226	173 (76.5%)	46 (20.4%)	7 (3.1%)	392 (86.7%)	60 (13.3%)	3.04	0.081
	Controls	266	166 (62.4%)	93 (35.0%)	7 (2.6%)	425 (80.0%)	107 (20.0%)	2.06	0.151
Baumber et al. - UK	Cases	63	41 (65.1%)	20 (31.7%)	2 (3.2%)	102 (81.0%)	24 (19.0%)	0.05	0.815
	Controls	89	57 (64.0%)	27 (30.3%)	5 (5.6%)	141 (79.2%)	37 (20.8%)	0.55	0.457
Baumber et al. - Denmark	Cases	96	56 (58.3%)	38 (39.6%)	2 (2.1%)	150 (78.1%)	42 (21.9%)	2.40	0.121
	Controls	72	37 (51.4%)	31 (43.1%)	4 (5.6%)	105 (72.9%)	39 (27.1%)	0.58	0.444
Baumber et al. - Sweden	Cases	98	68 (69.4%)	26 (26.5%)	4 (4.1%)	162 (82.7%)	34 (17.3%)	0.55	0.459
	Controls	106	67 (63.2%)	32 (30.2%)	7 (6.6%)	166 (78.3%)	46 (21.7%)	1.32	0.251
Meta-analysis	Cases	1167	792 (67.9%)	340 (29.1%)	35 (3.0%)	1924 (82.4%)	410 (17.6%)	0.04	0.838
	Controls	1618	1012 (62.6%)	533 (32.9%)	73 (4.5%)	2557 (79.0%)	679 (21.0%)	0.07	0.791

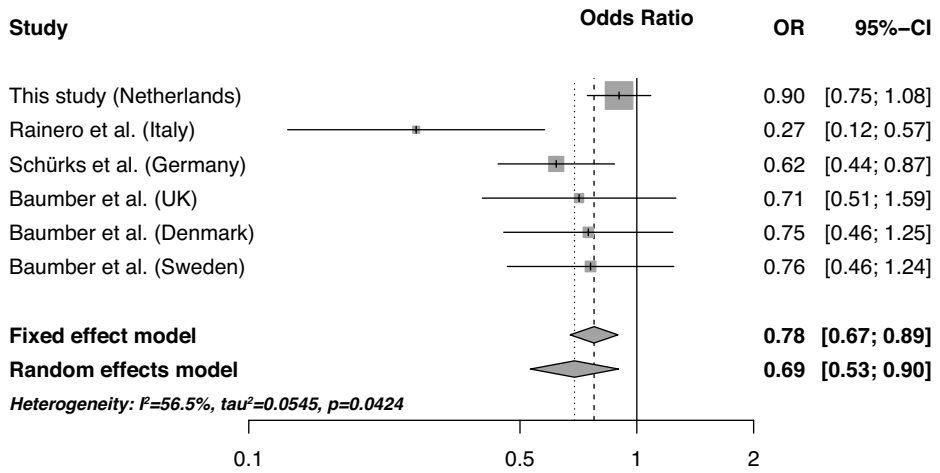

Figure 1 - Forest plot representing the results of the meta-analysis of the rs2653349 polymorphism of the *HCRTR2* gene assuming an additive model.

Results

A total of 587 participants from the LUCA study met our algorithm criteria[27] for cluster headache, provided blood for DNA isolation, and were included in the study. The majority of patients (73%) had the episodic form of the disorder and approximately 1 in 4 patients reported familial occurrence of cluster headache. Genotypes were obtained from 575 patients (clinical characteristics are summarized in Table 1) and 874 control subjects, and were in Hardy-Weinberg equilibrium (Table 2). No significant association between SNP rs2653349 and cluster headache was observed in our LUCA study (uncorrected OR: 0.90 (95% CI 0.75-1.08), p=0.265; corrected OR 0.91 (95% CI 0.75-1.10) p=0.319).

We identified 123 references meeting our aforementioned search criteria. Of these, a total of five studies reported on the association of SNP rs2653349 with cluster headache.[22-25,31] Three of these papers[22,25,31] described the same Italian study population and only the original research paper was included.[22] The Danish, Swedish and British populations from the study by Baumber et al.[23] were included as separate populations, leading to a total number of six study populations for our meta-analysis.

Subsequently, a meta-analysis was performed of three previously published studies (that included five study populations) and our own study.[22-24] These studies comprise in total 1,167 cluster headache patients and 1,618 control subjects. Genotype and allele frequencies of the various study populations are shown in Table 2. Genotype frequencies in the controls were in Hardy-Weinberg equilibrium in all studies. Our meta-analysis, assuming an additive model, replicated the previously reported association between the

Table 3 - Association with cluster headache in the study populations and the meta-analysis (additive model).

			Risk of cluster headache for carriers of A allele of rs2653349		Power for a given odds ratio				
Study	**Group**	**N**	**OR**	**p-value**	**OR 0.90**	**OR 0.80**	**OR 0.67**	**OR 0.50**	**OR 0.25**
This study - The Netherlands	Cases Controls	575 874	0.90 (0.75-1.08)	0.2646	0.16	0.43	0.97	1.00	1.00
Rainero et al. - Italy	Cases Controls	109 211	0.27 (0.12-0.57)	0.0003	0.07	0.15	0.37	0.77	1.00
Schurks et al. - Germany	Cases Controls	226 266	0.62 (0.44-0.87)	0.0055	0.06	0.18	0.53	0.98	1.00
Baumber et al. - UK	Cases Controls	63 89	0.71 (0.51-1.59)	0.7092	0.06	0.23	0.25	0.66	1.00
Baumber et al. - Denmark	Cases Controls	96 72	0.75 (0.46-1.25)	0.2694	0.06	0.12	0.30	0.73	1.00
Baumber et al. - Sweden	Cases Controls	98 106	0.76 (0.46-1.24)	0.2687	0.07	0.12	0.33	0.79	1.00
Meta-analysis	Cases Controls	1167 1618	0.69 (0.53-0.90)	0.0056	0.36	0.89	1.00	1.00	1.00

Power calculations were performed using a cluster headache prevalence of 0.02% with D' set to 1 (assuming perfect linkage disequilibrium of the marker allele and the disease allele) and equal prevalence of marker and high risk allele. Odds ratios are displayed below 1 because of the observed effect size of the A allele of rs2653349. The range of odds ratios used for the power calculations correspond to ORs of 1.11 – 1.25 – 2 – 4 for heterozygous carriers of the G allele.

A allele of the *HCRTR2* SNP and a decreased risk of cluster headache (random effect OR 0.69 (95% CI 0.53-0.90), p=0.006) (Figure 1)). The results of our meta-analysis indicated the presence of low to moderate between-study heterogeneity (I^2=0.57, p=0.042). Notably, the funnel plot graphically demonstrates asymmetry, indicating a publication bias favouring studies with an OR significantly deviating from 1, mainly caused by the initial study from Rainero *et al.*[22] (Figure 2). That study population is the only study from Southern Europe with allele frequencies that differ greatly from the other investigated populations that were all from Northern Europe. Remarkably, the absolute number of A alleles (8/218 alleles) among the cluster headache cases in the study population from Rainero *et al.* is very low compared to the other populations which makes it difficult to get reliable estimates of the allele frequency. We, therefore, performed an additional meta-analysis that contained only the populations from Northern Europe, so combining only data of Danish, Swedish, British, German and Dutch populations. The heterogeneity across these studies was negligible (I^2=0%, p=0.437), hence the random effect estimates correspond to the fixed effect estimates (OR 0.80 (95% CI 0.70-0.93), p=0.0031). Although the effect size decreased, the association remained.

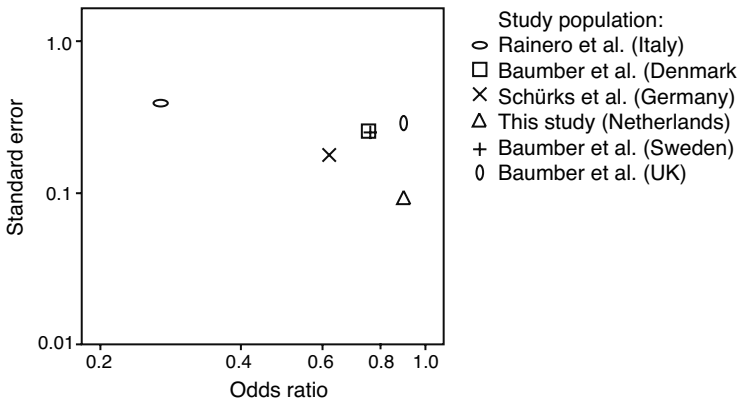

Figure 2 - Funnel plot of the six studies included in the meta-analysis of the rs2653349 (G1246A) polymorphism of the *HCRTR2* gene. The vertical axis shows the standard error of the log odds ratio for each study assuming an additive model, while the horizontal axis, the odds ratio, is again plotted in a log10 scale.

Discussion

Genetic factors have been implicated in cluster headache and several candidate gene association studies have been performed[16-21,22-24] aimed at identifying such factors but most studies did not produce convincing results, except for the genetic association with SNP rs2653349, which is located in the *HCRTR2* gene, that showed association for the (protective) A allele when using additive models. We decided to re-evaluate the association with this SNP by performing (i) a genetic association study in our LUCA population and (ii) meta-analyses of data from the LUCA study with data from earlier studies.

Our LUCA programme using validated questionnaire-based diagnoses[28] gave us the opportunity to include the largest number of cluster headache patients to date in a genetic study. Despite having adequate power (Table 3) to detect associations with similar effect sizes as reported by Rainero et al.[22] and Schürks et al. [24] no association (p=0.26) was found between the *HCRTR2* SNP rs2653349 and cluster headache. A subsequent meta-analysis of all selected previous studies together with the LUCA study revealed, however, a significant association of rs2653349 with cluster headache, but validity of the meta-analysis is questionable because all previously published studies had insufficient power due to small numbers of cases and controls (Table 3). The first study, by Rainero *et al.*[22], reported an association with a much larger effect than the other studies and seemed to drive the observed association effect. However, a second meta-analysis in which we removed that study from the analysis resulted in a large reduction of between-study heterogeneity with a slightly improved p-value for association (p=0.0031), albeit with a marked increase in

odds ratio (from 0.69 to 0.80). This indicates that the effect of the association is smaller when the first study is removed. Although our LUCA study *a priori* had by itself sufficient power to detect moderate effects on disease risk and has an acceptable power of 80% for detecting variants with an OR ≥ 1.31 (which is equivalent to OR ≤ 0.76 in case of a protective effect) (Table 3), it is underpowered to study genetic variants with small effect sizes that have been shown to underlie many genetic complex diseases.[26] Notably, a Cochrane review demonstrated that underpowered studies tend to have a large influence on the outcomes of meta-analyses if not at least two well-powered studies were included.[32] Moreover, a recent review, provocatively entitled "power failure: why small sample size undermines the reliability of neuroscience", addressed the important notion that low power not only decreases the chance to detect a true effect, but also decreases the chance that a significant finding from a small study reflects a true effect.[33] Such random fluctuations in allele frequencies may be driving the results of our meta-analyses. Thus, despite the apparent positive outcome of our meta-analyses, in our opinion, there is still no robust evidence for a true association between *HCRTR2* SNP rs2653349 and cluster headache.

In conclusion, our LUCA study and subsequent meta-analysis illustrate that even doubling of the sample size does not lead to a definite conclusion on the role of the *HCRTR2* gene in cluster headache. We demonstrated that such a role (if any) is likely smaller than previously reported. Future genetic studies in cluster headache patients need to include much larger numbers of patients and controls and may benefit also from focusing on hypothesis-free gene variant discovery, as is the case with GWAs.

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