Headache
© 2016 The Authors Headache published by
Wiley Periodicals, Inc. on behalf of American Headache Society

ISSN 0017-8748
doi: 10.1111/head.12807
Published by Wiley Periodicals, Inc.

# Research Submission

## Screening of Two *ADH4* Variations in a Swedish Cluster Headache Case–Control Material

Carmen Fourier, MSc; Caroline Ran, MSc, PhD; Anna Steinberg, MD, PhD;
Christina Sjöstrand, MD, PhD; Elisabet Waldenlind, MD, PhD; Andrea Carmine Belin, MSc, PhD

**Background.—Cluster headache (CH) is a severe neurovascular disorder and an increasing amount of evidence points to a genetic contribution to this disease. When CH was first described, it was observed that alcohol may precipitate an attack during the active phase of the disease. The alcohol dehydrogenase 4 (*ADH4*) gene encodes an enzyme which contributes to the metabolization of alcohol and is, therefore, an interesting candidate gene for CH. Two Italian groups have reported association of the single nucleotide polymorphism (SNP) rs1126671 located in the *ADH4* gene with an increased risk of CH in Italy. In addition, one of the groups found an association between the *ADH4* SNP rs1800759 and CH.**

**Objective.—To perform a replication study on the *ADH4* SNPs rs1126671 and rs1800759 in a large homogeneous Swedish case–control cohort in order to further investigate the possible contribution of *ADH4* to CH.**

**Methods.—A total of 390 unrelated patients diagnosed with CH and 389 controls representing a general Swedish population were recruited to the study. DNA samples from patients and controls were genotyped for the two *ADH4* SNPs rs1126671 and rs1800759 using quantitative real-time polymerase chain reaction. Statistical analyses of genotype, allele and haplotype frequencies for the two SNPs were performed and compared between patients and controls.**

**Results.—For rs1126671, the minor allele frequency (A allele) was 32.8% (n = 254) in controls compared with 31.9% (n = 249) in CH patients. The minor allele frequency (A allele) of rs1800759 was 42.3% (n = 324) in controls and 41.9% (n = 327) in CH patients. Statistical analysis showed no significant differences in allele as well as in genotype or haplotype frequencies between the patient and control group for either SNP. This was also seen after stratifying the patient group for experiencing alcohol as a trigger factor.**

**Conclusions.—The data did not support an association of the *ADH4* SNPs rs1126671 and rs1800759 with CH. A comparison with previous studies revealed variance in genotype, allele, and haplotype frequencies among the different populations which might contribute to the contradictory results. Although a significant association with CH in Swedish case–control group was not found, *ADH4* as a candidate gene for CH could not be excluded.**

**Key words: haplotype, polymorphism, alcohol**

**Abbreviations: ADH4 alcohol dehydrogenase 4, CH cluster headache, SNP single nucleotide polymorphism**

(*Headache* 2016;56:835-840)

**From the Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden (C. Fourier, C. Ran, and A. Carmine Belin); Department of Clinical Neuroscience, Karolinska University Hospital, Stockholm, Sweden (A. Steinberg, C. Sjöstrand, and E. Waldenlind).**

**Address all correspondence to A. Carmine Belin, Department of Neuroscience, Karolinska Institutet, 171 77 Stockholm, Sweden.**

**Accepted for publication January 11, 2016.**

## INTRODUCTION

Cluster headache (CH), also known as Horton's headache, is a severe neurovascular disease characterized by an intense, unilateral head pain and is generally accompanied by autonomic symptoms, such as swelling of the eye, tearing, nasal congestion, or a sense of restlessness. Attacks occur in episodic bouts, lasting weeks or months, separated by remission periods of months to years. About 10%–15% of the patients suffer from a chronic subtype where remission lasts less than 1 month/year.[1] CH has a prevalence of up to 0.1%,[2] and is three times more common in men than in women.[1] An increasing number of studies show a genetic contribution to CH, and 7%-20% of the patients have a positive family history, defined as having at least one first-, second-, or third-degree relative also diagnosed with CH.[3,4] The causes of CH and those factors that might trigger the onset of an active period are still unknown. However, already in 1941 Bayard Horton observed that alcohol may elicit an attack in CH patients during the active phase.[5] More recently, it has been reported that 52%-79% of the patients experience alcohol to trigger an attack when in a bout.[6-9] However, the study reporting a frequency of 79% included only male patients with episodic CH.[6] Among others, this observation has led to investigations on genes for CH susceptibility which are involved in alcohol metabolism. One family of enzymes with a primary role in the breakdown of alcohol is the alcohol dehydrogenase (ADH) family. They are predominantly expressed in the liver as well as gastrointestinal tract,[10,11] and catalyze the oxidation of alcohols, such as ethanol or retinol. There are several subunits of ADH encoded by different genes. Specifically *ADH4*, which is located on chromosome 4q22–23, has been implicated in a number of diseases and traits, for instance, Parkinson's disease,[12] alcohol and drug dependence,[13,14] personality traits such as extraversion and agreeableness,[15] and moreover it has been associated with CH.[16,17] The single nucleotide polymorphism (SNP) rs1126671 located in exon 7 of the *ADH4* gene was associated with an increased risk for CH in two Italian studies, whereas rs1800759 located in the promoter region of *ADH4* could only be associated with CH in the smaller case–control study.[17] The rs1126671 SNP induces an amino acid change from isoleucine to valine in the corresponding protein. It is hypothesized that this amino acid change could produce altered enzyme functionality by possibly interfering with an adjoining phosphorylation site.[16] For the *ADH4* SNP rs1800759, it has been demonstrated that promoter activity in hepatoma cells is drastically increased in the mutant allele and could therefore alter ADH4 $\pi$-subunit expression.[18] In expression quantitative trait loci (eQTL) analysis using the Gtex Portal (http://www.gtexportal.org/home/), both rs1126671 and rs1800759 have been found to have a significant correlation to the transcript levels of *ADH4*. In esophagus muscularis tissue, the risk alleles (A for both SNPs) were associated with higher *ADH4* expression levels, while in fibroblast cells the risk alleles were associated with lower *ADH4* expression levels (data sources GTEx Analysis Release V6; dbGaP Accession phs000424.v6.p1).

In other genetic association studies, opposing results have pointed to the presence of geographical and ethnical differences that underline the importance of validating findings in independent, homogeneous cohorts. Thus, we performed a replication study on the *ADH4* SNPs rs1126671 and rs1800759 in a large, Swedish case–control cohort in order to further investigate the possible contribution of *ADH4* to CH.

## MATERIAL AND METHODS

The study cohort consisted of 390 CH patients and 389 controls (Table 1). The study material was obtained after approval of the local ethics committee in Stockholm and informed consent of both patients and controls. CH patients were recruited from the neurology clinic at the Karolinska University Hospital, Stockholm, Sweden. The diagnosis of CH was based on the International Classification of Headache Disorders (ICHD-III) criteria.[1] The majority of the controls (n = 379) were anonymous healthy blood donors from the Stockholm area recruited when donating blood at the Karolinska University Hospital. This group

*Conflict of Interest*: None.

**Table 1.—Demographic and Clinical Characterization of CH Patients and Control Subjects**

| | CH Cases | Controls |
|---|---|---|
| Total number of subjects | 390 | 389 |
| Men (%) | 270 (69.2) | 208 (53.5) |
| Mean age ± SD in years | 53.5 ± 14.5 | NA |
| Mean age of onset ± SD in years | 31.2 ± 13.5 | |
| Patients with chronic CH (%) | 39 (10.0) | |
| Positive family history†—n (%) | 40/349 (11.5) | |
| Alcohol trigger: Yes—n (%) | 196/349 (56.2) | |

NA = not available; n = fraction of patients who filled out questionnaire; SD = standard deviation.
†At least one first-, second-, or third-degree relative with CH diagnosis.

represents a general healthy Swedish population, with no demographic information available except for gender. Ten additional controls were neurologically healthy individuals recruited when visiting the neurology clinic at the Karolinska University Hospital. Blood samples were collected from CH patients during 2000 and 2014–2015 and from control individuals during 2005 and 2015. DNA was extracted using standard protocols. The majority of patients (n = 349) as well as 10 neurologically healthy controls completed a questionnaire regarding clinical data, medication, and lifestyle. Genotyping was performed by TaqMan® quantitative real-time polymerase chain reaction (7500 fast system using standard cycling conditions, TaqMan® genotyping master mix and Genotyping Assay C_11941799_30 for rs1126671 or C_8829281_20 for rs1800759; Applied Biosystems, Carlsbad, CA). The data on the two *ADH4* SNPs for all 779 individuals were exclusively collected for this replication study.

**Statistical Analyses.—**Genotype association was evaluated with chi-square ($\chi^2$) test and allele association was analyzed with Fisher's exact test using GraphPad Prism v5.04 (GraphPad Softwares Inc, La Jolla, CA, USA). Additionally, genotypic association was tested for via logistic regression with sex as covariate using PLINK whole genome association analysis toolset v1.07.[19] A possible difference in the proportions of subgroups of the patient cohort regarding alcohol as a trigger factor was tested for by using a two-tailed two-proportion Z-test. All statistical tests were performed at significance level 0.05. Haplotype analysis was performed in HaploView v.4.2.[20] For power analysis, the power and sample size software PS v3.0[21] was used. With our sample size and a minor allele frequency of 0.33 (http://www.ncbi.nlm.nih.gov/projects/SNP/), we are able to detect true odds ratios (OR) for CH below 0.64 or above 1.51, respectively. The observed frequencies of controls were in agreement with the Hardy–Weinberg equilibrium which was tested for using the web-based Online Encyclopedia for Genetic Epidemiology studies software.[22]

## RESULTS

We analyzed the two *ADH4* SNPs rs1126671 and rs1800759 in a total of 390 CH patients and 389 controls, and tested for association between each of the polymorphisms and CH. Comparing genotype as well as allele frequencies of CH patients with

**Table 2.—Genotype and Allele Frequencies of Two *ADH4* Polymorphisms in Control Subjects and CH Patients**

| SNP | Genotype | Controls (%) | CH Cases (%) | $\chi^2$ (df) | *P*-Value | Allele | Controls (%) | CH Cases (%) | OR (95% CI) | *P*-Value |
|---|---|---|---|---|---|---|---|---|---|---|
| rs1126671 | GG | 173 (44.7) | 176 (45.1) | 0.418 | .811 | G | 520 (67.2) | 531 (68.1) | 0.96 | .745 |
| | GA | 174 (45.0) | 179 (45.9) | (2) | | A | 254 (32.8) | 249 (31.9) | (0.78–1.19) | |
| | AA | 40 (10.3) | 35 (9.0) | | | | | | | |
| rs1800759 | CC | 123 (32.1) | 128 (32.8) | 0.047 | .977 | C | 442 (57.7) | 453 (58.1) | 0.98 | .918 |
| | CA | 196 (51.2) | 197 (50.5) | (2) | | A | 324 (42.3) | 327 (41.9) | (0.80–1.21) | |
| | AA | 64 (16.7) | 65 (16.7) | | | | | | | |

*ADH4* = alcohol dehydrogenase 4; $\chi^2$ = chi-square; df = degrees of freedom; OR = odds ratio; CI = confidence interval. Genotype association was evaluated with a $\chi^2$ test and allele association was analyzed with Fisher's exact test.

**Table 3.—Genotype and Allele Frequencies of Two *ADH4* Polymorphisms in Control Subjects and CH Patients That Reported Alcohol as an Attack Trigger**

| SNP | Genotype | Controls (%) | $CH_{alctrigger}$ (%) | $\chi^2$ (df) | *P*-Value | Allele | Controls (%) | $CH_{alctrigger}$ (%) | OR (95% CI) | *P*-Value |
|---|---|---|---|---|---|---|---|---|---|---|
| rs1126671 | GG | 173 (44.7) | 85 (43.4) | 0.402 | .818 | G | 520 (67.2) | 263 (67.1) | 1.00 | 1.000 |
| | GA | 174 (45.0) | 93 (47.4) | (2) | | A | 254 (32.8) | 129 (32.9) | (0.78–1.09) | |
| | AA | 40 (10.3) | 18 (9.2) | | | | | | | |
| rs1800759 | CC | 123 (32.1) | 60 (30.6) | 0.141 | .932 | C | 442 (57.7) | 223 (56.9) | 1.03 | .802 |
| | CA | 196 (51.2) | 103 (52.6) | (2) | | A | 324 (42.3) | 169 (43.1) | (0.81–1.32) | |
| | AA | 64 (16.7) | 33 (16.8) | | | | | | | |

*ADH4* = alcohol dehydrogenase 4; $CH_{alctrigger}$ = CH patients that report alcohol as a trigger factor; $\chi^2$ = chi-square; df = degrees of freedom; OR = odds ratio; CI, confidence interval. Genotype association was evaluated with a $\chi^2$ test and allele association was analyzed with Fisher's exact test.

controls for each SNP did not reveal a significant difference between the case and control group (Table 2). Logistic regression with sex as a covariate was run in order to exclude any bias introduced by the skewed sex ratio in the patient group. We could not find a genotypic association with CH for either rs1126671 ($P = .753$) or rs1800759 ($P = .985$) when applying sex-corrected logistic regression. To take into account variability in subgroups of patients, we additionally performed a stratified analysis with respect to whether the patient regarded alcohol as a trigger factor or not (information retrieved from the questionnaire). When comparing controls with only patients that have reported alcohol to trigger an attack (n = 196; 56.2% of patients who filled out the questionnaire), there were no significant differences in the genotypes of the two groups for either SNP (Table 3). Furthermore, we did the following observations from the patients who answered the questionnaire: 58.1% of the male (n = 236) and 52.2% of the female (n = 113) CH patients reported alcohol as a trigger factor. In addition, 55.7% of the episodic (n = 309) and 60.5% of the chronic (n = 38) CH patients experienced alcohol to elicit an attack. There were no significant differences in the proportions of male and female, or episodic and chronic, patients who reported alcohol as a trigger factor (data available upon request). Finally, a haplotype analysis was carried out for the two *ADH4* polymorphisms (Table 4); none of the haplotypes were associated with CH.

## DISCUSSION

This is the largest study on *ADH4* and CH reported so far. Genotypic and allelic analyses of the investigated *ADH4* polymorphisms rs1126671 and rs1800759 in CH patients suggest that there is no association between these genetic variants and CH in Sweden. The power of this study is much higher compared with previous studies where an association between *ADH4* and CH has been found.[16,17] The two previously reported studies have been carried out on comparably small cohorts including only 110 and 54 CH patients, respectively. However, it is possible that population differences may lead to these varying results. Genotype and allele frequencies observed in controls from our study roughly correspond to the ones reported in

**Table 4.—Haplotype Analysis for Two *ADH4* Polymorphisms in Control Subjects and CH Patients**

| Haplotype | Controls (%) | CH Cases (%) | $\chi^2$ (df = 1) | *P*-Value |
|---|---|---|---|---|
| G–C† | 435 (57.0) | 454 (57.9) | 0.117 | .732 |
| A–A | 244 (32.0) | 250 (31.5) | 0.042 | .838 |
| G–A | 79 (10.4) | 80 (10.3) | 0.005 | .943 |
| A–C | 4 (0.6) | 2 (0.3) | 0.635 | .426 |

$\chi^2$ = chi-square; df = degrees of freedom.
†Reference haplotype (G–C) corresponds to wild-type allele of each *ADH4* single nucleotide polymorphism where rs1126671 is in position 1 and rs1800759 in position 2.

the two Italian studies. Yet, for rs1800759 the mutant allele seems to be less common in the Italian cohorts (31.0%–33.7%) compared with our Swedish cohort (42.3%). From our questionnaire data, 56.2% of the patients reported that alcohol may trigger an attack which corresponds to what other groups have reported (52%–63%).[7-9] When performing a statistical analysis for control individuals and CH patients reporting alcohol as a trigger factor, there were no significant differences in the genotypes of the two groups for either SNP. A comparison between the haplotype analysis from one of the Italian studies[16] and our Swedish analysis emphasizes the deviation in haplotype frequencies among these two populations. For example, the diplotype A–C, corresponding to the mutated allele of rs1126671 and the wild-type allele of rs1800759, is very rare in our Swedish material (0.6%), whereas in the Italian cohort (27.9%) it is much more common.

In conclusion, our data suggest that the SNPs rs1126671 and rs1800759 in the *ADH4* gene are not genetic risk factors for CH in the Swedish population. Although we did not find a significant association with CH in our Swedish case–control material, we could not exclude *ADH4* as a candidate gene for CH. More studies on additional *ADH4* SNPs as well as novel candidate genes will be needed to answer the question why and how alcohol may precipitate attacks during a bout in more than half of the patients with CH.

*Acknowledgments: We thank Fengqing Xiang for excellent technical assistance and Ann-Christin Karlsson for help with recruitment of patients. This work was supported by the Swedish Brain Foundation, Swedish Research Foundation, Åke Wibergs Stiftelse, Swedish Brain Power, Svenska Migränsällskapet, and Karolinska Institutet Research funds.*

## STATEMENT OF AUTHORSHIP

**Category 1**

**(a) Conception and Design**

Carmen Fourier, Caroline Ran, Andrea Carmine Belin

**(b) Acquisition of Data**

Carmen Fourier, Anna Steinberg, Christina Sjöstrand, Elisabet Waldenlind

**(c) Analysis and Interpretation of Data**

Carmen Fourier, Caroline Ran, Andrea Carmine Belin

**Category 2**

**(a) Drafting the Manuscript**

Carmen Fourier, Andrea Carmine Belin

**(b) Revising It for Intellectual Content**

Carmen Fourier, Caroline Ran, Anna Steinberg, Christina Sjöstrand, Elisabet Waldenlind, Andrea Carmine Belin

**Category 3**

**(a) Final Approval of the Completed Manuscript**

Carmen Fourier, Caroline Ran, Anna Steinberg, Christina Sjöstrand, Elisabet Waldenlind, Andrea Carmine Belin

## REFERENCES

1. Headache Classification Committee of the International Headache Society. The International Classification of Headache Disorders, 3rd edition (beta version). *Cephalalgia.* 2013;33:629-808.
2. Leroux E, Ducros A. Cluster headache. *Orphanet J Rare Dis.* 2008;3:20.
3. Russell MB, Andersson PG, Thomsen LL. Familial occurrence of cluster headache. *J Neurol Neurosurg Psychiatry.* 1995;58:341-343.
4. Leone M, Russell MB, Rigamonti A, et al. Increased familial risk of cluster headache. *Neurology.* 2001;56:1233-1236.
5. Horton BT. The use of histamine in the treatment of specific types of headaches. *JAMA.* 1941;116: 377-383.
6. Levi R, Edman G V, Ekbom K, Waldenlind E. Episodic cluster headache II: High tobacco and alcohol consumption in males. *Headache.* 1992;32: 184-187.
7. Bahra A, May A, Goadsby PJ. Cluster headache: A prospective clinical study with diagnostic implications. *Neurology.* 2002;58:354-361.
8. Schürks M, Kurth T, Knorn P, Pageler L, Diener HC. Cluster headache: Clinical presentation, lifestyle features, and medical treatment. *Headache.* 2006;46:1246-1254.

9. Rozen TD, Fishman RS. Cluster headache in the United States of America: Demographics, clinical characteristics, triggers, suicidality, and personal burden. *Headache.* 2012;52:99-113.
10. Edenberg HJ. Regulation of the mammalian alcohol dehydrogenase genes. *Prog Nucleic Acid Res Mol Biol.* 2000;64:295-341.
11. Uhlén M. Tissue-based map of the human proteome. *Science.* 2015;347:1260419-1260419.
12. Buervenich S, Sydow O, Carmine A, Zhang Z, Anvret M, Olson L. Alcohol dehydrogenase alleles in Parkinson's disease. *Mov Disord.* 2000;15: 813-818.
13. Guindalini C, Scivoletto S, Ferreira RG, et al. Association of genetic variants in alcohol dehydrogenase 4 with alcohol dependence in Brazilian patients. *Am J Psychiatry.* 2005;162:1005-1007.
14. Luo X, Kranzler HR, Zuo L, Yang BZ, Lappalainen J, Gelernter J. ADH4 gene variation is associated with alcohol and drug dependence: Results from family controlled and population-structured association studies. *Pharmacogenet Genom.* 2005;15:755-768.
15. Luo X, Kranzler HR, Zuo L, Wang S, Gelernter J. Personality traits of agreeableness and extraversion are associated with ADH4 variation. *Biol Psychiatry.* 2007;61:599-608.
16. Rainero I, Rubino E, Gallone S, et al. Cluster headache is associated with the alcohol dehydrogenase 4 (ADH4) gene. *Headache.* 2010;50:92-98.
17. Zarrilli F, Tomaiuolo R, Ceglia C, et al. Molecular analysis of cluster headache. *Clin J Pain.* 2015;31:52-57.
18. Edenberg HJ, Jerome RE, Li M. Polymorphism of the human alcohol dehydrogenase 4 (ADH4) promoter affects gene expression. *Pharmacogenetics.* 1999;9:25-30.
19. Purcell S, Neale B, Todd-Brown K, et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81:559-575.
20. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005;21:263-265.
21. Dupont WD, Plummer WD. Power and sample size calculations. A review and computer program. *Control Clin Trials.* 1990;11:116-128.
22. Rodriguez S, Gaunt TR, Day INM. Hardy-Weinberg equilibrium testing of biological ascertainment for mendelian randomization studies. *Am J Epidemiol.* 2009;169:505-514.