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Sequence Variants in Host Cell Factor C1 Are Associated With Ménière's Disease

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Abstract

Hypothesis—There is a genetic basis for the development of Ménière's (MD) disease.

Background—The cause of MD is unknown, although many potential theories have been proposed. A genetic basis for the disease is suggested by greater prevalence in Caucasians and familial cases that display an autosomal dominant pattern of inheritance.

Methods/Design—Case-control association study of selected candidate genes among patients with MD and selected control individuals.

Results—Several single-nucleotide polymorphisms (SNPs) within the host cell factor C1 (*HCFC1*) gene displayed a significant increase in prevalence of the major allele in subjects with MD disease. The most individually significant SNP is rs2266886. The minor allele at this site displays an odds ratio of 0.26 (95% confidence intervals, 0.010–0.65; $p = 0.003$) for disease development.

Conclusion—The minor allele at each SNP site was significantly more common in controls, suggesting that individuals bearing these alleles are at reduced risk of developing MD. The functional consequences of the SNPs in host cell factor C1 are unknown. A viable hypothesis for disease development is presented based on the known interaction between *HCFC1* and the herpes simplex viral protein VP16.

Keywords

Ménière's disease; Herpes simplex virus; Host cell factor C1

Ménière's disease (MD) is a clinical disorder of cochleovestibular dysfunction of unknown cause. The disorder is typically of adult onset, with a mean age at presentation of between 45 and 55 years of age. The prevalence in the United States population is approximately 1:1,000 (1). Individuals of European descent develop the disorder more frequently than other ethnic groups. Men and women are affected with equal frequency. The first familial cases of MD were reported by Brown in 1941 (2). It is estimated that 8% of all MD patients have at least 1 affected family member (2–4). There is limited information available on the genetics of the disease. Analysis of some MD pedigrees suggests an autosomal dominant mode of inheritance with incomplete penetrance (4–7). However, it cannot be ruled out that MD is a complex disease due to the interactions of multiple genes and environmental factors. Siblings of MD patients display a 10-fold increased risk of developing the disease (3). Association with other diseases

is also speculated because some recognize a higher prevalence of migraine and autoimmune diseases in MD patients (4,7,8).

Early attempts to identify genetic factors associated with MD examined human leukocyte antigens; however, no consistent associations were found. Renewed speculation of a genetic marker for MD followed the identification of *COCH* as the causative gene in DFNA9, a syndrome of familial hearing loss and vestibular dysfunction. Some investigators suggested the clinical symptoms of MD and DFNA9 were similar, and that *COCH* was a reasonable candidate gene for MD (9). However, subsequent studies did not reveal any potentially functional *COCH* sequence variants in subjects with MD (10).

There are a few reports of candidate genes studied for association with MD selected on the basis of physiology. Aquaporin 2 (*AQP2*) is hypothesized to play a role in endolymph homeostasis and is found in the endolymphatic sac. Mhatre et al. (11) sequenced *AQP2* in 12 MD patients, but no potentially functional variants were detected. Doi et al. (12) selected 2 potassium channel genes, *KCNE1* and *KCNE3*, for study predicated on the hypothesis that ion channel dysfunction may contribute to MD. A single-nucleotide polymorphism (SNP) was tested in each gene, and both showed significant allele frequency differences. The SNP rs1805127 in *KCNE1* is also studied in this report, but an association with MD is not confirmed. Finally, Klar et al. (13) identified a candidate gene, phosphoinositide 3 kinase (*PIK3C2G*), through linkage analysis of cases of familial MD. The area of linkage in the families they studied contained only the *PIK3C2G* gene; however, fine mapping could not identify any SNPs or mutations that showed association with MD in the families.

Many theories of pathogenesis of MD have been proposed, including viral infection or reactivation, migraine variant, autoimmune disorder, vascular compromise, allergy, and altered endolymph circulation. None of these theories are universally accepted. Not surprisingly, the medical treatment of MD is widely varied and of dubious efficacy (14,15). There is abundant information implicating genetic predisposition to migraine, autoimmune diseases, allergy, and even susceptibility to infectious diseases; thus, it is possible, if not likely, that a genetic contribution to MD development exists. In this report, the results of a candidate gene case-control association study are reported. Single-nucleotide polymorphisms were genotyped in multiple genes that were selected based on a suspected role in MD pathogenesis. The study provides evidence that variants within the host cell factor C1 (*HCFC1*) gene play a role in the cause of MD.

METHODS

Subjects

Before onset, the study was approved by the institutional review board of Baylor College of Medicine. Informed consent was secured from all individuals who agreed to participate in the research. Inclusion as a case subject required fulfillment of criteria for definite MD according to guidelines established by the Hearing and Equilibrium Committee of the American Academy of Otolaryngology–Head and Neck Surgery were enrolled in the study (16). The criteria for definite MD require a history of characteristic vertigo episodes, documentation of low-frequency sensorineural hearing loss, and associated symptoms of tinnitus and aural pressure. All case subjects underwent cranial magnetic resonance imaging examination to further exclude neoplasm or demyelinating disease. Control specimens consisted of banked DNA samples, most of which were obtained from the Baylor Polymorphism Resource (<http://www.cardiogene.org>). Medical history was unavailable for the controls. All cases and controls are European Americans.

DNA Isolation

Blood was collected from cases and controls by peripheral venipuncture. Lymphoblastoid cell lines were established using conventional Epstein-Barr virus-mediated transformation protocols. DNA was isolated from transformed lymphocytes using the PUREGENE DNA purification kit (Gentra Systems, Inc., Minneapolis, MN, USA) for cultured cells according to the manufacturer's specifications.

Candidate Gene Selection

The genes selected for SNP analysis were selected according to several theories of the pathogenesis of MD (17). Included were genes involved in herpes simplex virus (HSV) cellular entry, viral transcription, and reactivation; mediators of inflammation; and genes implicated in familial migraine, familial cochleovestibular dysfunction, or potassium ion transport within the stria vascularis. A total of 69 SNPs in 39 genes were studied. Single-nucleotide polymorphism coverage within a given gene was not uniform. For each of the candidate genes, SNPs were selected based on physical distance (usually >5 kb between SNPs), minor allele frequency (average, 27%; range, 5–56%), and, when available, haplotype data from the International HapMap Project. (<http://www.hapmap.org>).

SNP Genotyping

Commercially available validated SNP assays were used for genotyping (Applied Biosystems, Inc., Foster City, CA, USA). This technique uses a duplex fluorescence polymerase chain reaction (PCR) using differentially labeled TaqMan probes for the major and minor alleles of each SNP. The change in fluorescence of each reporter from baseline to endpoint and amplification plot are used to assign genotype. Reactions were run on an ABI 7000 real-time PCR machine (Applied Biosystems). Standardized reaction mixtures (1 × universal master mix, 1 × primer/probe mix, internal positive control, and 5 ng of template DNA in each 25-μl reaction) and cycling parameters (initial denaturization at 95°C for 10 min, and then 40 cycles of 92°C for 15 s, 60°C for 60 s) are used for each SNP assay.

Data Analysis

Each SNP was tested for Hardy-Weinberg equilibrium (HWE) using the exact test in cases and controls separately for all the SNPs on the autosomes. Next, the Holman Freeman Fisher's exact test and the Cochran-Armitage trend test were used to analyze if there was a difference in the genotype frequencies between cases and controls. For those SNPs located on the X chromosome to avoid confounding due to the sex of the study participants, individuals with zero variants were collapsed into 1 group, and those with either 1 or 2 variants were collapsed into another group according to case-control status, and the Fisher's exact test was used to analyze the resulting 2 × 2 tables. The genotypes for multiple SNPs that were in the same genes or neighboring genes were analyzed using Fisher's product method, and empirical *p* values were obtained with permutation tests using 5,000 replicates. Statistical tests were performed using the R statistical package (R Core Development Team, 2007). Haplotype analysis was performed examining SNPs within single genes and neighboring genes. Haplotypes were constructed using the UNPHASED program, and a likelihood ratio test was performed (18). Linkage disequilibrium within chromosomal regions was evaluated by examining r^2 values. The UNPHASED program allows for haplotype reconstruction using the EM algorithm for both markers on the autosomes and those marker loci on the X chromosome. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using the Sheehe correction.

DNA Sequencing

The DNA sequence for the coding regions of *HCFC1* is administered by Genbank reference sequence accession numbers X84221 (exon 1) and X79198 (exons 2–26) (19). All primers

used for the PCR amplification and sequencing were designed de novo and can be provided on request. Polymerase chain reaction products were resolved by gel electrophoresis to confirm the expected size. Post-PCR purification used ExoSAP-IT 78201 (USB, Corp., Cleveland, OH, USA) according to manufacturer's guidelines. Initial sequencing was completed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). Electropherograms were analyzed and interpreted using Mutation Surveyor software, version 2.41 (Softgenetics, Inc., State College, PA, USA).

Reverse-Transcriptase-PCR

Two cell lines homozygous for the sequence variation and 2 wild-type cell lines were selected for reverse-transcriptase (RT)-PCR to analyze if an exon is deleted in the presence of the variant. RNA was extracted from lymphocyte cell lines using Versagene RNA purification kit (Gentra Systems) according to the manufacturer's guidelines. The RT-PCR reaction used a Fidelity Taq RT-PCR kit (USB Corp). Each reaction consisted of the master mix, 100 ng of template RNA, and 15 pmol of primers. DNA primers were set at the 3' end of exon 3 and the 5' end of exon 6 to produce a 412-base pair product spanning all of exons 4 and 5. Primer sequences are forward, 5'-AACATTCCAAGGTACCTGAATG-3' and reverse, 5'-GCCATGGTATCCAGGTTGAG-3'. The PCR product was resolved on a 1% Agarose gel.

RESULTS

Initial analysis of 60 SNPs was conducted using a series of 21 cases and 33 controls. Six SNPs were excluded from analysis, 4 SNPs were not in HWE in controls using a criterion of $p \leq 0.05$, and 2 SNPs were monomorphic. Additional cases and controls were added, and the statistics were recalculated. The number of additional cases varied according to magnitude of preliminary genotype or allele frequency differences. In almost all cases, additional data produced a decrease in the value of the test statistics. Nine additional SNPs were added to the survey at a later date, and preliminary analysis was conducted using a different set of 30 cases and 40 controls. A list of genes considered and SNPs tested is provided in Tables 1 and 2.

Based upon nominal p values, significant results were found for several SNPs (Table 3). For SNP rs1049216 (185925238 bp), which is located in the *CASP3* (chr4, 185,924,000–185,945,777) gene, a significant finding was found only for the Holman-Freeman-Fisher's exact test ($p = 0.000035$). For this SNP, there was a deviation from HWE in controls ($p = 0.07$) with an excess of heterozygote genotypes (disequilibrium coefficient, -0.046).

Significant results were found for 4 flanking SNPs on the X chromosome: rs2269372 (152728392 bp), rs762653 (152739767 bp), rs17421 (152746481 bp), and rs2266886 (152752199 bp). These SNPs, which are in linkage disequilibrium with each other, are located in the *RENBP* (chr23, 152,721,569–152,731,078) gene and the *HCFC1* gene (chr23, 152,733,854–152,751,023). The Fisher's exact test was significant for the SNPs in the *HCFC1* gene: rs762653 ($p = 0.015$), rs17421 ($p = 0.004$), and rs762653 ($p = 0.015$) and rs2266886 ($p = 0.023$) in the *RENBP* gene. Directly telomeric to the *HCFC1* gene is SNP rs2266887 (152760434 bp), which is located in the predicated open reading frame cXorf12 (chr23, 152,759,087–152,769,486), and there was significant evidence of an association with MD. The Fisher product method was used to evaluate the significance of the 3 SNPs within the *HCFC1* gene (empiric nominal $p = 0.0014$). Haplotype analysis with all combinations of SNPs within the *HCFC1* produced statistically significant results (data not shown). Linkage disequilibrium was observed between the SNPs with the *HCFC1* gene with higher levels of linkage disequilibrium in controls as compared with cases. For each of the SNPs in the *HCFC1* gene, a higher frequency of the rare allele was observed in controls compared with cases. The ORs for the SNPs within the *HCFC1* gene are for rs762653 (OR, 0.33 95% CI, 0.13–0.81); rs17421 (OR, 0.21; 95% CI, 0.07–0.65); rs2266886 (OR, 0.26; 95% CI, 0.10–

0.65). For each OR calculated, the reference group was those individuals who had 1 or 2 copies of the minor allele.

Sequence Analysis

A subset of 10 cases and 10 controls underwent resequencing of the coding exons and adjacent intronic sequences of *HCFC1*. The sequenced regions included 17 coding and 19 noncoding SNPs. No minor alleles were detected for 11 of the cSNPs and 17 of the noncoding SNPs that have been previously reported. The SNPs with genotype differences are listed in Table 4. Only 1 of the SNPs, rs1051152, produces a change in amino acid sequence. Sequencing also detected a previously unreported SNP that is labeled in the Celera database as hCV25623002. This SNP produces a C>T change 13 bases upstream from the first nucleotide of exon 5 in the polypyrimidine tract binding site. A total of 33 cases and 72 controls were genotyped for this SNP by resequencing. The prevalence of the minor allele was 8% in cases and 25% in controls. In men, the prevalence was 0% in cases and 20% in controls, whereas in women, the prevalence was 13 and 28%, respectively. No female cases were homozygous for the minor allele. The OR for presence of the T allele at hCV25623002 and MD is 0.27 (95% CI, 0.10–0.76).

RT-PCR Analysis

The location of hCV25623002 in the 3' splice site of intron 4 suggests a possible effect on splicing. There are 2 known isoforms of *HCFC1* in normal tissue, although the alternative splice site occurs in exon 8 (20). An experiment was designed to test whether the hCV25623002 T allele impacts splicing of exon 5. RNA was isolated from transformed lymphoblasts. Cell lines were chosen based upon genotype at the IVS4–13 position. RNA was extracted from 2 cell lines homozygous for the T allele, 2 heterozygotes, and 2 with the wild-type C allele. Exon skipping was not observed in the specimens with the T allele (Fig. 1). To confirm that no splicing errors occurred that could not be detected on agarose gel electrophoresis, sequencing of the RT-PCR products was conducted in the forward and reverse directions. DNA sequencing revealed proper splice junctions between exons 4, 5, and 6, suggesting that the T allele does not affect splicing in lymphocytes.

DISCUSSION

In this study, several SNPs were identified that are in disequilibrium with MD. Multiple SNPs within *HCFC1* were identified, suggesting *HCFC1* can be related to MD pathogenesis. The only SNP that was not within or near *HCFC1* was rs1049216, located within the *CASP3* gene. The data obtained do not strongly support an association between rs1049216 and MD because this SNP was statistically significant only for the Holman-Freeman-Fisher's exact test and not the Cochran-Armitage test for trend. Within controls, the HWE exact test produced $p = 0.07$, with a disequilibrium coefficient of -0.046 indicating an excess of heterozygotes. An adjacent SNP, rs1049253, which is in strong linkage disequilibrium with rs1049216, was rejected for having significant Hardy-Weinberg disequilibrium in controls. It is possible that the observed findings in *CASP3* are due to chance or genotyping error. Therefore, our study does not provide enough evidence to support an association between *CASP3* and MD. In contrast, the evidence of association between SNPs in *HCFC1* and MD is compelling. To date, there is no known disease associated with altered activity of *HCFC1*, but a logical pathway for disease development can be construed based on the known functions of the gene, specifically its interaction with herpes viruses.

HCFC1 is involved in a wide variety of cellular functions, including regulation of transcription, cytokinesis, cell cycle progression, and chromatin remodeling (21). The protein is essential for cellular viability and demonstrates similar activity among a broad range of species. *HCFC1* is expressed in all tissues, although quantitatively, levels are lower in neural tissue (22).

Subcellular location of the protein is uniquely within the cytoplasm in neurons, whereas it is found in the nuclei of other cell types. Interestingly, transport of *HCFC1* to the cell nucleus in neurons is associated with reactivation of HSV (23).

The protein was initially described due to its interaction with HSV viral protein VP16 and *POU2F1* (Oct-1), forming the VP16-induced complex (VIC). In permissive cells, formation of the VIC results in induction of HSV immediate early (IE) gene synthesis and subsequent viral replication (24). *HCFC1* stabilizes the VIC and functions as a coactivator of transcription. Studies of Oct-1–deficient cells find that there are alternative pathways for VP16-induced HSV IE gene transcription. However, depletion of intracellular *HCFC1* severely impairs HSV IE gene expression, indicating the essential contribution of *HCFC1* to this process (25). In addition, expression of varicella zoster virus IE genes is also critically dependent on *HCFC1*. The identification of *HCFC1* as a key element in directing viral synthesis in the host cell was the basis for its inclusion as a candidate gene in this study.

The *HCFC1* precursor protein is 2,035 amino acids. Posttranscriptional cleavage of the precursor protein forms separate amino- and carboxy-terminal fragments that remain noncovalently associated. The amino terminal contains a 6-bladed β propeller, termed the Kelch domain, which is composed of 6 repeat sequences with significant similarity to the *Drosophila* Kelch protein. The Kelch domain binds VP16 and several other transcription factors, including *CREB3*, *HPIP*, and Zhangfei through recognition of a shared tetrapeptide HCF1 binding motif (HBM). Alteration of the HBM adversely affects interaction between *HCFC1* and VP16. Mutation analysis confirms that all blades of the β -propeller are important for VP16 binding. Preferential recognition of *HCFC1* by VP16 compared with the related protein HCFC2 is due to alterations in HCF Kelch 5 in the latter (26). Differential effects on *CREB3* and VP16 association were observed in several engineered mutants, indicating that sequences adjacent to the HBM are also important for specific recognition of VP16 (27).

A single amino acid substitution (P134S) in the Kelch domain in the BHK21 hamster cell line tsBN67 is responsible for a temperature-dependent arrest in cell cycle progression (28). In the hyperthermic state, *HCFC1*-chromatin association is disrupted, leading to arrest of cell cycle progression in the G1/G0 phase. Normal cell cycle progression occurs on return to the permissive temperature or by addition of wild-type *HCFC1*. The amino terminal residues 1–902 have been shown to be critical for tsBN67 rescue. Other mutations in the Kelch domain impair the ability of *HCFC1* to rescue arrested tsBN67 cells, indicating that a critical factor in cell cycle progression also binds to this region of *HCFC1*. The P134S substitution also impairs VP16 binding, suggesting that the virus evolved to mimic the interaction of a protein involved in cell cycle progression (29).

The possibility of HSV reactivation as a causative factor in MD has been reviewed in previous work (30). Initial speculation was based on similarity in clinical course between MD and other HSV-mediated diseases. Transient symptoms interspersed between long symptom-free intervals are features of MD, herpes labialis, and ocular herpes. A necessary prerequisite for the theory of HSV reactivation would be demonstration of HSV DNA in the vestibular ganglion. A study of archival vestibular ganglia from MD patients undergoing vestibular neurectomy confirmed the universal presence of HSV DNA (30). A high prevalence of HSV in control ganglia predicted that infection alone was an inadequate explanation for development of symptoms, and that host response to the virus was important. Further work displayed a wide variation of HSV viral load in cranial nerve ganglia, thus reducing the likelihood that reactivation is solely a manifestation of high viral load in a given ganglion (31).

Although the functional consequences of the *HCFC1* SNPs are not known, the association of *HCFC1* with VP16 suggests a plausible role for *HCFC1* variants in the modulation of HSV

latency in infected cells. In neurons, *HCFC1* is rapidly transported to the nucleus under “stress” conditions that provoke viral reactivation. The demonstration of reduced HSV transcription efficiency in *HCFC1*-depleted cell lines suggests that disease mediated by HSV reactivation can be enhanced in individuals with greater cellular levels of *HCFC1* or reduced in those with impaired *HCFC1* activity (25). If any sequence changes in *HCFC1* reduce its transcriptional activity or result in low affinity association with VP16, the probability of HSV reactivation would be reduced in infected cells. The latter mechanism would be predicted to have less effect on other cellular functions of *HCFC1*. The mechanism of action may also be tissue specific, affecting only latency within sensory neurons. In this scenario, a host is not immune to initial HSV infection and may manifest characteristic epithelial ulcerations but does not display reactivation due to altered *HCFC1* function in neurons.

In summary, the results of this study provide evidence in support of the hypothesis that development of MD is at least partially due to genetic factors. The finding of linkage disequilibrium between sequence variations in *HCFC1* and MD predicts this gene may be involved in disease pathogenesis. Based on known functions of *HCFC1*, concurrent herpes virus infection is a likely necessary cofactor, and reactivation of latent virus can be the mechanism underlying production of cochleovestibular symptoms. There are several alternative explanations for the observations described. First, the sequence variations in *HCFC1* are not related to MD pathogenesis, but rather, are in strong linkage disequilibrium with the true functional variants within the *HCFC1* gene or a neighboring gene. Second, the association that was detected was due to population admixture in that the controls and the cases were drawn from different populations with different allele frequencies for the SNPs studied in the *HCFC1* gene. Finally, it is possible the association between *HCFC1* and MD might be a false positive and will not be confirmed in future studies. It is clear that additional population screening and functional assessment are needed to ascertain the validity of association between *HCFC1* sequence variations and MD. Confirmation of any gene purported to play a role in MD requires elucidation of the mechanism of cochleovestibular dysfunction that is characteristic of MD or reproduction of the symptoms in an animal model.

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References

1. Wladislavosky-Waserman P, Facer GW, Mokri B, Kurland LT. Ménière's disease: a 30-year epidemiologic and clinical study in Rochester, Mn, 1951–1980. *Laryngoscope* 1984;94:1098–102. [PubMed: 6611471]
2. Brown MR. Ménière's syndrome. *Arch Neurol Psychiatry* 1941;46:561–5.
3. Morrison AW. Ménière's disease. *J Laryng Otol* 1995;109:499–502.
4. Morrison AW, Johnson KJ. Ménière's disease. *Otolaryngol Clin North Am* 2002;35:497–516. [PubMed: 12486836]
5. Birgerson L, Gustavson KH, Stahle J. Ménière's disease: a genetic investigation. *Am J Otol* 1987;8:323–6. [PubMed: 3631240]
6. Fung K, Xie Y, Hall SF, Lillicrap DP, Taylor SA. Ménière's disease. *J Otolaryngol* 2002;31:1–4. [PubMed: 11883436]
7. Oliveira CA, Ferrari I, Messias CI. Ménière's syndrome and migraine in Brasilia. *Ann Otol Rhinol Laryngol* 2002;111:229–36. [PubMed: 11913683]

8. Ruckenstein MJ, Prasthoffer A, Bigelow DC, Von Feldt JM, Kolasinski SL. Ménière's disease. *Otol Neurotol* 2002;23:517–20. [PubMed: 12170155]
9. Fransen E, Verstreken M, Verhagen WIM, et al. Ménière's disease in three families with a mutation in the *COCH* gene. *Hum Molec Genet* 1999;8:1425–9. [PubMed: 10400989]
10. Usami S, Takahashi K, Yuge I, et al. Mutations in the *COCH* gene are a frequent cause of autosomal dominant progressive cochleovestibular dysfunction, but not of Ménière's disease. *Eur J Hum Genet* 2003;11:744–8. [PubMed: 14512963]
11. Mhatre AN, Jero J, Chiappini I, Bolasco G, Barbara M, Lalwani AK. Aquaporin-2 expression in the mammalian cochlea and investigation of its role in Ménière's disease. *Hear Res* 2002;170:59–69. [PubMed: 12208541]
12. Doi K, Sato T, Kuramasu T, et al. Ménière's disease is associated with single nucleotide polymorphisms in the human potassium channel genes, *KCNE1* and *KCNE3*. *ORL J Otorhinolaryngol Relat Spec* 2005;67:289–93. [PubMed: 16374062]
13. Klar J, Frykholm C, Friberg U, Dahl N. A Ménière's disease gene linked to chromosome 12p12.3. *Am J Med Genet B Neuropsychiatr Genet* 2006;141:463–7. [PubMed: 16741942]
14. Ruckenstein MJ, Rutka JA, Hawke M. Ménière's disease: Torok revisited. *Laryngoscope* 1991;101:211–8. [PubMed: 1992276]
15. Grant IL, Welling DB. Ménière's disease. *Otolaryngol Clin North Am* 1997;30:1123–44. [PubMed: 9386248]
16. Committee on Hearing and Equilibrium guidelines for the diagnosis and evaluation of therapy in Ménière's disease. *Otolaryngol Head Neck Surg* 1995;113:181–5. [PubMed: 7675476]
17. Merchant SN, Rauch SD, Nadol JB Jr. Ménière's disease. *Eur Arch Otorhinolaryngol* 1995;252:63–75. [PubMed: 7598874]
18. Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* 2003;25:115–21. [PubMed: 12916020]
19. Zoppe M, Frattini A, Faranda S, Vezzoni P. The complete sequence of the host cell factor 1 (*HCFC1*) gene and its promoter: a role for YY1 transcription factor in the regulation of its expression. *Genomics* 1996;34:85–91. [PubMed: 8661027]
20. Wilson AC, Lamarco K, Peterson MG, Herr W. The VP16 accessory protein HCF is a family of polypeptides processed from a large precursor protein. *Cell* 1993;74:115–25. [PubMed: 8392914]
21. Khurana B, Kristie TM. A protein sequestering system reveals control of cellular programs by the transcriptional coactivator HCF-1. *J Biol Chem* 2004;279:33673–83. [PubMed: 15190068]
22. Wilson AC, Parrish JE, Massa HF, Nelson DL, Trask BJ, Herr W. The gene encoding the VP16-accessory protein HCF (*HCFC1*) resides in human Xq28 and is highly expressed in fetal tissues and the adult kidney. *Genomics* 1995;25:462–8. [PubMed: 7789979]
23. Kristie TM, Vogel JL, Sears AE. Nuclear localization of the C1 factor (host cell factor) in sensory neurons correlates with reactivation of herpes simplex virus from latency. *Proc Natl Acad Sci U S A* 1999;96:1229–33. [PubMed: 9990006]
24. Wysocka J, Herr W. The herpes simplex virus VP16-induced complex: the makings of a regulatory switch. *Trends Biochem Sci* 2003;28:294–304. [PubMed: 12826401]
25. Narayanan A, Nogueira ML, Ruyechan WT, Kristie TM. Combinatorial transcription of herpes simplex virus and varicella zoster virus immediate early genes is strictly determined by the cellular coactivator HCF-1. *J Biol Chem* 2005;280:1369–75. [PubMed: 15522876]
26. Johnson KM, Mahajan SS, Wilson AC. Herpes simplex virus transactivator VP16 discriminates between HCF-1 and a novel family member, HCF-2. *J Virol* 1999;73:3930–40. [PubMed: 10196288]
27. Mahajan SS, Wilson AC. Mutations in host cell factor 1 separate its role in cell proliferation from recruitment of VP16 and LZIP. *Mol Cell Biol* 2000;20:919–28. [PubMed: 10629049]
28. Goto H, Motomura S, Wilson AC, Freiman RN, Nakabeppu Y, Fukushima K, et al. A single-point mutation in HCF causes temperature-sensitive cell-cycle arrest and disrupts VP16 function. *Genes Dev* 1997;11:726–37. [PubMed: 9087427]
29. Freiman RN, Herr W. Viral mimicry: common mode of association with HCF by VP16 and the cellular protein LZIP. *Genes Dev* 1997;11:3122–7. [PubMed: 9389645]
30. Vrabec JT. Ménière's disease. *Laryngoscope* 2003;113:1431–8. [PubMed: 12972911]

31. Vrabec JT, Alford RL. Quantitative analysis of herpes simplex virus in cranial nerve ganglia. *J Neurovirol* 2004;10:216–22. [PubMed: 15371151]

FIG. 1.

Exon 5 splicing in lymphocytes. *Lane 1*, base ladder; *lane 2*, hemizygous T; *lane 3*, heterozygote CT; *lane 4*, homozygous TT; *lane 5*, hemizygous C; *lane 6*, heterozygote; *lane 7*, homozygous CC; *lane 8*, blank.

TABLE 1

Candidate genes

Function	Gene/protein
HSV entry/transcription	<i>HCFC1</i> , POU2F1, CREB3, ZF, PVRL1, PVRL2
HSV susceptibility/reactivation	NGFB, <i>CASP3</i> , CNTF, IL6, STAT1, TRPV1, TNF, IFNG, CDK2, TNFRSF1A, TNFRSF1B
Inflammatory signaling (locus)	STAT3, STAT5, NFKB1, NR2C2, ICAM1, CCL5 (POLB, MGC33648)
Familial migraine (locus)	CACNA1A, ATP1A2 (CDKN3, IL10)
Genes within human genome correlates of mouse HSV susceptibility loci	<i>JAK1</i> , <i>BCL11B</i> , <i>IL9</i> , <i>HTR2A</i>
Familial cochleovestibular dysfunction	<i>COCH</i> , POU3F4
K ⁺ transport within stria vascularis	SLC12A2, ATP1A1, ATP1B2, <i>KCNE1</i>

HSV indicates herpes simplex virus.

TABLE 2

List of SNPs tested

rs10089	rs1570248	rs2266887	rs3869550
rs1045644	rs1598857	rs2269372	rs395908
rs1049216	rs1641512	rs2280232	rs4142495
rs1049253	rs17421	rs2292293	rs474247
rs1053023	rs1800629	rs2293152	rs550942
rs1135669	rs1805127	rs2302350	rs5922814
rs1152781	rs1860545	rs266818	rs6330
rs1152793	rs1862263	rs266819	rs6571366
rs11670018	rs1914408	rs281432	rs702680
rs1192	rs1923882	rs2953983	rs762653
rs12117962	rs1978782	rs310198	rs769178
rs1405937	rs1991923	rs310228	rs773108
rs1407130	rs2069718	rs31564	rs790154
rs1422259	rs2069845	rs367264	rs850609
rs1467199	rs2179896	rs3764580	rs910330
rs1497862	rs2193049	rs3773480	
rs1534847	rs222747	rs3774964	
rs1554286	rs2266886	rs3817655	

SNP indicates single-nucleotide polymorphism.

TABLE 3

SNP sites showing association with MD

SNP ID	Gene	MAF Case	Control	FET	CATT	OR
rs1049216	<i>CASP3</i>	0.32	0.27	3.5e10-5 ^a	0.47	4.33 (1.47–12.80) ^b 0.38 (0.17–0.85) ^c
hCV25623002	<i>HCFC1</i>	0.08	0.24	0.014	NA	0.27 (0.1–0.76)
rs2266886	<i>HCFC1</i>	0.10	0.29	0.003	NA	0.26 (0.1–0.65)
rs17421	<i>HCFC1</i>	0.06	0.24	0.004	NA	0.21 (0.07–0.65)
rs762653	<i>HCFC1</i>	0.09	0.21	0.015	NA	0.33 (0.13–0.81)
rs2269372	<i>RENBP</i>	0.14	0.25	0.023	NA	0.38 (0.17–0.85)

^aHolman-Freeman-Fisher's exact test.

^bHomozygous for the common allele used for the reference group, OR was calculated comparing the reference group to heterozygotes.

^cHomozygous for the common allele used for the reference group, OR was calculated comparing the reference group to homozygotes for the minor allele.

CATT indicates Cochran-Armitage test for trend; FET, Fisher's exact test; MAF, minor allele frequency; NA, not applicable; OR, odds ratio (and the 95% confidence interval calculated using the Sheehe correction).

TABLE 4

Sequenced HCFC1 SNPs

SNP ID	Location	Case MAF	Control MAF
rs2071134	Exon 13	0.07	0.40
rs3027878	Exon 19	0.07	0.40
rs730106	Exon 16	0.13	0.47
rs1051152	Exon 17	0.13	0.47
rs2071133	Exon 17	0.13	0.47
rs2071132	Intron 23	0.13	0.47
rs3027875	Exon 24	0.67	0.40

MAF indicates minor allele frequency; SNP, single-nucleotide polymorphism.