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Chromosome 19p13 Loci in Finnish Migraine With Aura Families

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Abstract

Chromosomal area 19p13 contains two migraine associated genes: a $Ca_v2.1$ (P/Q-type) calcium channel α_1 subunit gene, *CACNA1A*, and an insulin receptor gene, *INSR*. Missense mutations in *CACNA1A* cause a rare Mendelian form of migraine, familial hemiplegic migraine type 1 (FHM1). Contribution of *CACNA1A* locus has also been studied in the common forms of migraine, migraine with (MA) and without aura (MO), but the results have been contradictory. The role of *INSR* is less well established: A region on 19p13 separate from *CACNA1A* was recently reported to be a major locus for migraine and subsequently, the *INSR* gene was associated with MA and MO. Our aim was to clarify the role of these loci in MA families by analyzing 72 multigenerational Finnish MA families, the largest family sample so far. We hypothesized that the potential major contribution of the 19p13 loci should be detected in a family sample of this size, and this was confirmed by simulations. We genotyped eight polymorphic microsatellite markers surrounding the *INSR* and *CACNA1A* genes on 757 individuals. Using parametric and non-parametric linkage analysis, none of the studied markers showed any evidence of linkage to MA either under locus homogeneity or heterogeneity. However, marginally positive lod scores were observed in three families, and thus for these families the results remain inconclusive. The overall conclusion is that our study did not provide evidence of a major MA susceptibility region on 19p13 and thus we were not able to replicate the *INSR* locus finding.

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Keywords

INSR; *CACNA1A*; linkage; migraine with aura

INTRODUCTION

One of the most intriguing regions with involvement in migraine is the chromosome 19p13, which contains two migraine associated genes, *INSR* and *CACNA1A*. This region has mostly been associated with a rare Mendelian form of migraine, familial hemiplegic migraine (FHM1), caused by *CACNA1A* mutations [Ophoff et al., 1996]. However, recently Jones et al. reported that this region and specifically insulin receptor gene (*INSR*) would be a major locus for common forms of migraine, especially migraine with aura [Jones et al., 2001].

The only true molecular insight into migraine pathophysiology has been provided by the identification of FHM mutations. By contrast, the molecular background of more common forms of migraine, migraine with (MA) and without (MO) aura, has remained largely unresolved. Recently, a number of susceptibility loci has been detected in genome-wide scans, but no underlying variants have been identified [Carlsson et al., 2002; Wessman et al., 2002; Bjornsson et al., 2003; Cader et al., 2003; Soragna et al., 2003]. The involvement of *CACNA1A* in more common forms of migraine has been suggested, the hypothesis being that the pathophysiology of common and rare forms of migraine shares same cellular pathways. Convincing molecular evidence for this is still missing, but it is encouraging that both *CACNA1A* encoding the α_1 subunit of neuronal $\text{Ca}_v2.1$ (P/Q-type) calcium channels and the other gene identified to cause FHM, *ATP1A2* encoding Na^+, K^+ -ATPase α_2 subunit, are involved in ion transport of the cell membrane [De Fusco et al., 2003]. This idea of similar underlying pathophysiology has stimulated a number of studies testing the contribution of *CACNA1A* (FHM1) locus in both MA and MO. Some of these studies have reported suggestive linkage to the FHM1 locus [May et al., 1995; Nyholt et al., 1998; Terwindt et al., 2001]. Also, several contradictory studies exist where no linkage or association to this locus was found in MA or MO [Hovatta et al., 1994; Lea et al., 2001; Noble-Topham et al., 2002]. In addition, a few groups have reported sequencing either the entire *CACNA1A* gene or selected areas of it in patients with common forms of migraine but no mutations have been found [Lea et al., 2001; Brugnani et al., 2002; Wieser et al., 2003].

Based on the evidence reported by Jones et al. [2001], the chromosomal region of 19p13 containing the *INSR* gene would have a major contribution to migraine susceptibility. According to their data, 13 out of the 16 MA families studied were linked to this region flanked by markers D19S427 and D19S592 [Jones et al., 2001]. Subsequently, they showed that five single nucleotide polymorphisms (SNPs) within the *INSR* gene were significantly associated with migraine [McCarthy et al., 2001]. These findings should trigger replication studies.

Based on previous studies, the involvement of the chromosome 19p13 in common forms of migraine remains suggestive with only one study [Jones et al., 2001] suggesting a major

contribution of this locus in 16 North-American families. Since in our genome-wide scan [Wessman et al., 2002] one of the several lod scores with a nominal *P*-value of 0.05 was observed with marker D19S427 close to the *INSR*, the contribution of the two chromosome 19p loci in MA was further studied in 72 multigenerational MA families from our Finnish migraine family cohort. We hypothesized that if either the *INSR* or the *CACNA1A* locus contributes to MA, this would be detectable in our large sample.

MATERIALS AND METHODS

Families

Since 1996, our group has recruited MA families with at least three affected first-degree family members from four Finnish headache clinics (Helsinki, Turku, Jyväskylä, and Kemi) for clinical and molecular genetic studies. Additionally, advertisements in the newsletter of the Finnish Migraine Society were used. Thus, this migraine sample represents families all over Finland rather than a specific subisolate. The recruitment of the families is described in more detail elsewhere [Wessman et al., 2002]. Diagnosis of the index cases and family members were made by one neurologist (MK) according to the IHS criteria [International Headache Society, 1988] on the basis of the validated Finnish Migraine-Specific Questionnaire for Family studies. The questionnaire contains 37 detailed questions about headache and other aspects of migraine with special emphasis on the characteristics of aura [Kallela et al., 2001]. Individuals with incompletely or contradictory filled questionnaires were interviewed by telephone by the same neurologist.

For this study, we selected the most complete 72 independent multigenerational families with a seemingly dominant inheritance pattern. Fifty out of these 72 families were genotyped in the genome-wide linkage analysis demonstrating that MA is linked to chromosome 4q24 [Wessman et al., 2002]. Blood samples from 757 (446 females, 311 males) out of a total of 978 family members were available including 417 individuals with MA and 91 individuals with MO. Table I describes the diagnosis of the 757 genotyped subjects. Most of the DNA samples were received from families with three generations (41 families out of 72). Of the remaining families, one was four-generational, 27 were two-generational, and three one-generational. The ethics committees of the Helsinki University Central Hospital and the University of California, Los Angeles approved this study and an informed consent was obtained from all family members.

Clinical characteristics of the MA patients.—The aura phase of the affected subjects was dominated by visual symptoms, but also other types of migraine aura were reported. Aura was visual in 381 (91.4%) cases, a speech disturbance in 128 (30.7%), sensory in 150 (36.0%), and motor in 71 (17.0%) cases. Motor aura was of hemiparetic nature in 68 subjects. Visual aura was a scintillating scotoma in 228 (57%), visual field defect in 173 (43.5%), and photopsia in 196 (49.1%) cases. Additional blurring of vision was reported by 182 patients (45.6%). The headache phase was characterized by frequent and disabling attacks. The number of attacks was over 100 in 38.4%, 50–100 in 22.3%, 10–50 in 22.3%, 5–10 in 6.5%, and 2–5 in 1.2% of the MA patients. Thirty subjects had repeated attacks but did not state their exact lifetime frequency. Headache phase lasted usually less than 4 hr in

19.2%, 4–72 hr in 74.6%, and more than 72 hr in 5.3% of the subjects. Four patients did not indicate the duration of their attacks. 383 patients could recall accurately the onset of their migraine and in this group the mean age of onset was 14.3 years (SD 7.4). IHS-defined characteristics [International Headache Society, 1988] of the headache are presented in supplementary Table I.

Genotyping

Genotyping was performed on 757 individuals from 72 MA families with eight polymorphic microsatellite markers D19S247, D19S427, D19S592, D19S391, D19S394, D19S221, D19S1150, and D19S226 surrounding the *INSR* and the *CACNA1A* genes. Figure 1 shows the locus order and the genetic map distances of the genotyped markers. The PCR fragments, along with a size standard ladder and fragments from known control individuals (CEPH) were analyzed on ABI3700 DNA analyzer (Applied Biosystems, Foster City, CA) using the GeneScan and Genotyper Software (Applied Biosystems). All genotypes were verified by human inspection and reviewed if the PedCheck1.1 [O'Connell and Weeks, 1998] and SimWalk2 2.82 [Sobel and Lange, 1996] computer programs detected genotyping errors. Remaining problematic genotypes were set to unknown.

Statistical Analyses

The analyses were performed assuming autosomal dominant inheritance, disease-gene frequency of 0.001, a phenocopy rate of 0.024, and an incomplete penetrance of 0.9 [Wessman et al., 2002]. Allele frequencies were estimated from all genotypes. Only individuals with MA were treated as affected and all other subjects were classified as unknown (affected-only strategy).

Two-point linkage analyses were performed both under locus homogeneity using the MLINK program of the Linkage package [Lathrop and Lalouel, 1984] and allowing for locus admixture using the HOMOG program [Ott, 1991]. In addition, the identity-by-descent (IBD) status of the affected sib-pairs (ASP) was analyzed to investigate the possibility that a putative migraine gene may act in a recessive fashion. Additionally, nonparametric linkage (NPL) analysis was performed using the SimWalk2 version 2.82 [Sobel and Lange, 1996] to investigate the robustness of the results independently of the assumed genetic model. In NPL analysis, the statistical significance of alleles shared IBD between all affected family members is estimated. SimWalk's statistics B (for dominant traits) and E (NPL_all) were considered.

In order to evaluate how informative the pedigrees are for linkage, simulation studies were used. A marker with five alleles of equal frequency (heterozygosity 0.8) was generated completely linked ($\theta = 0$) to the disease locus. For the simulation study, the disease allele frequency and penetrance model is the same that was used to analyze the data. For each study, a total of 500 replicates was generated using the program SLINK [Ott, 1989; Weeks et al., 1994]. The data was then analyzed using MSIM [Ott, 1989; Weeks et al., 1994] and ELODHET [Ott, 1991], a program that applies the locus admixture test [Ott, 1983; Smith, 1963]. Two measures were evaluated, the expected maximum lod score (EMLOD) and power. The approximation to the EMLOD is the average of the maximum lod scores for

each replicate regardless of the theta value at which it occurred. The power, the probability under linkage that the null hypothesis of no linkage will be rejected, is the proportion of replicates where the lod score is equal to or greater than a given threshold (lod 3.0 under linkage homogeneity, lod 3.3 in the presence of linkage admixture) [Terwilliger and Ott, 1994]. In order to determine the power to detect linkage in the presence of admixture, the α (proportion of families linked to the disease locus) was varied from 1.0 to 0.1 in increments of 0.1. In addition, for those families, which produced a lod score >0.59 for at least one of the markers, the EMLOD and maximum lod score were estimated.

RESULTS

Two-Point Linkage Analyses

Two-point linkage analyses were performed in 72 MA families using an affected-only analysis and a narrow affection status criteria by treating only MA patients as affected. The information content of the studied markers varied between 68 and 92% being the highest at D19S394. None of the eight genotyped markers showed any evidence for linkage to the studied 34 cM region surrounding the *INSR* and the *CACNA1A* genes on 19p13 either under homogeneity or heterogeneity (Table II). The highest lod score of 0.16 under heterogeneity (HLOD) at a recombination fraction of 0.14 was observed with marker D19S394. The lod scores of the intragenic marker D19S1150 of *CACNA1A* provided no evidence for linkage in these Finnish MA families (HLOD 0.00). Neither of the *INSR* flanking markers, D19S427 and D19S592, provided evidence of linkage (HLOD 0.07 and 0.00, respectively). The combined two-point lod scores varied between -26.9 and -46.1 at the recombination fraction (θ) of 0.00 assuming homogeneity (Table II). Furthermore, none of the ASP scores showed evidence of linkage to the genotyped region (Table II). When the affection status was broadened to include also MO ($n=91$) individuals, the highest lod score was obtained with marker D19S391 (LOD 0.13, $\theta = 0.46$) both under locus homogeneity and heterogeneity.

We carried out a series of simulations of linkage in the 72 families using the SLINK, MSIM, and ELODHET programs. The EMLOD and the power to detect linkage with different values of α are shown in supplementary Figure 1. The results showed that for this data set, it should be possible to detect linkage even when only 30% of the families are linked to the same locus (power = 0.7; EMLOD=4.7). When $<30\%$ of the families are linked, the power drops quickly. However, even in the case of only 10% of the families being linked, the EMLOD is still slightly above 1.0, which is clearly higher than the highest maximum lod score achieved in the present study.

The contribution of individual families was also studied by ranking all families according to their lod scores. Figure 3 (see the online Figure 3 at <http://www.interscience.wiley.com/jpages/0148-7299:1/suppmat/index.html>) shows the distribution of individual families' lod scores for the four markers, D19S427, D19S592, D19S394, and D19S1150 surrounding both the *INSR* and *CACNA1A* genes, at a recombination fraction 0.00. The number of families having LOD scores >0.3 , >0.5 , and >1.0 were 11, 5, and 1 for D19S427, 3, 1, and 0 for D19S592, 5, 5, and 1 for D19S394 and 5, 2, and 0 for D19S1150.

Ten families out of the 72 displayed lod scores at or above 0.59, which is associated with a nominal P -value of 0.05 [Lander and Kruglyak, 1995], in any recombination fraction to at least one of the studied markers (supplementary Table II). The highest lod score for marker D19S427 identifying the *INSR* region was 1.15 ($\theta=0$) in family number 86. The markers at the *CACNA1A* region, D19S394, D19S221, D19S1150, and D19S226 showed lod scores of 1.30 ($\theta=0.05$, family 103), 0.96 ($\theta=0$, family 16), 0.59 ($\theta=0$, family 128), and 1.14 ($\theta=0$, family 95), respectively. However, the results of the flanking markers were much lower in all cases (see the online Table IV at <http://www.interscience.wiley.com/jpages/0148-7299/1/suppmat/index.html>). The simulated EMLOD and maximum lod scores for these 10 families are also reported in Table IV. It should be noted that due to the reduced penetrance and phenocopies, the simulated maximum lod scores shown may never be obtained, even in the case where the disease and the marker loci are completely linked and the marker is fully informative. Only one of these families (128) is potentially large enough to establish linkage by itself. This family produces only a slightly positive lod score suggesting that it is probably not linked to this region. Due to the size of the other pedigrees it is difficult to conclude whether the migraine locus segregating in them maps to this region. Yet the finding, that in each family only one marker provided a lod score of >0.59 makes this hypothesis unlikely. When comparing the actual and the simulated results, it can be seen that four out of ten families showed a lod score at or near the simulated maximum. The haplotype segregation in these four families was analyzed and in three families, all MA patients shared a common haplotype within a family.

Non-Parametric Linkage Analysis

We also performed non-parametric linkage analysis with SimWalk2 to exclude the effect of model specification to linkage analysis. The NPL scores did not support linkage (Table II). The statistic B of SimWalk2 showed the highest $-\log_{10}(P)$ value of 0.79 ($P=0.16$) for marker D19S394.

DISCUSSION

The recently reported MA susceptibility locus on 19p13 [Jones et al., 2001] and the association of migraine with the *INSR* gene [McCarthy et al., 2001] are intriguing findings that called for replication studies. In the present study, however, the *INSR* flanking markers D19S427 and D19S592 provided no evidence for linkage (HLOD scores 0.07 and 0.00, respectively) to migraine. Thus we were not able to replicate the nominal linkage to marker D19S427 (1.70, $\theta=0.22$) that was observed in our genome wide screen [Wessman et al., 2002]. Such low lod scores are often false positives and the information provided by the 22 additional families are likely to explain these results. In the present study, the highest individual family lod score for marker D19S592 was 0.57 ($\theta=0.00$). For the other *INSR* flanking marker, D19S427, a lod of 1.15 ($\theta=0.00$) was observed with family number 86. Simulation studies indicate that this is the highest lod score, which can be obtained for this family. Haplotype analysis showed, that all affected individuals within family 86 shared a common haplotype extending from marker D19S247 to D19S391. Due to the size of the pedigree, it is difficult to conclude whether this single family has a predisposing allele in this region.

We did expect to find at least nominal evidence of linkage to the *INSR* region considering the very high proportion of linked families (estimated $\alpha=0.99$) in the study of Jones et al. [2001]. Based on our simulation results, the power of our study to detect linkage is high (power 69%, EMLOD 4.7) even in the case of only 30% of the families being linked. Since the families included in the study of Jones et al. are all North American Caucasians of mainly western or northern European descent [Jones et al., 2001], it seems unlikely that the ethnicity would be the only cause of this discrepancy. It is also worth noting that the IHS criteria for migraine [International Headache Society, 1988] have been used in both cases. Thus gross differences in disease classification are unlikely to explain the discrepancy between our findings and the results by Jones et al. [2001]. Such a discrepancy between loci identified in different study samples is not a rare event in complex traits (e.g. hypertension and schizophrenia [O'Donovan et al., 2003; Mein et al., 2004]). The underlying reasons are not entirely clear but factors like different ascertainment, population origin, and details in diagnostic procedures are likely to contribute to heterogeneity.

Furthermore, our linkage analysis showed no evidence of linkage to D19S1150, the intragenic marker of *CACNA1A*. The maximum HLOD score was 0.00 and the highest lod score for an individual family (family 128) 0.59 ($\theta=0$). This is in accordance with the previous study on four Finnish MA/MO families [Hovatta et al., 1994] and also with the results of our genome-wide screen [Wessman et al., 2002]. None of our 72 MA families or the 64 Canadian MA families studied by Noble-Topham et al. [2002] showed even suggestive evidence for linkage to the *CACNA1A* region. In addition, the results of the linkage and association studies performed on 82 Australian MA/MO pedigrees were negative [Lea et al., 2001]. Interestingly, sequencing of *CACNA1A* in two patients belonging to the only published MA family with suggestive linkage to this region [Nyholt et al., 1998] showed no disease causing mutations [Lea et al., 2001]. Thus, *CACNA1A* seems not to be a major susceptibility locus for the common forms of migraine in different populations although there still may be high penetrance alleles contributing to the phenotype in selected families. Evidence is also mounting that this locus is not even a minor predisposing locus for migraine but even larger study samples are required to confirm this.

The clinical characteristics of our 72 families are very comparable to the results of a population based study of MA reported by Russell et al. [1996]. There are slightly more sensory and motor auras and slightly less visual auras (90 vs. 99%) in our families compared to Russell's figures, but overall the similarities clearly outweigh the differences. The fairly large prevalence of hemiparetic and hemisensory symptoms is of note, because based on the present study genes in chromosome 19 are unlikely to cause them.

In conclusion, although several positive findings linking migraine with aura to chromosome 19p13 have been published, we find that in 72 Finnish MA families, the largest data set used so far, neither *INSR* nor *CACNA1A* locus shows evidence for linkage to this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

The locus order of genotyped markers and the localization of the *INSR* and the *CACNA1A* genes. Information on marker order and genetic map distances was obtained from the UCSC (<http://genome.ucsc.edu/>, July 2003 release), the Genome Database (<http://www.gdb.org>), and the Marshfield for Medical Genetics (<http://research.marshfieldclinic.org/genetics/>) databases. Both the genetic and the physical distance between markers is reported (cM = centiMorgan, Mb = Megabase).

TABLE I.

Summary of the Genotyped Individuals From the 72 Pedigrees

Gender	Trait						Total/gender
	MA	MO	EQV	HA	NoHa	MD	
Male	116	35	3	13	119	25	311
Female	301	56	5	10	51	23	446
Total/trait	417	91	8	23	170	48	757

MA, migraine with aura; MO, migraine without aura; EQV, migraine aura without headache; HA, headache; NoHa, no headache; MD, missing diagnosis.

TABLE II.

Two-Point Lod Scores and NPL Scores for the Eight Studied Markers

Marker	Results under locus homogeneity		Results under locus heterogeneity		Lod scores of ASP analysis		Load at $\theta = 0.00$		NPL_all (SimWalk stat E)	
	Max lod	θ	Max lod	0					$-\log(P)$	P-value
D19S247	0.00	–	0.00	–		0.01	–45.24		0.27	0.54
D19S427	0.00	–	0.07	0.00		0.00	–26.86		0.63	0.23
D19S592	0.00	–	0.00	–		0.01	–38.71		0.71	0.19
D19S391	0.03	0.46	0.03	0.46		0.00	–39.86		0.52	0.30
D19S394	0.00	–	0.16	0.14		0.00	–43.80		0.79	0.16
D19S221	0.00	–	0.00	–		0.00	–44.38		0.36	0.44
D19S1150	0.00	–	0.00	–		0.00	–44.65		0.44	0.36
D19S226	0.00	–	0.00	–		0.00	–46.06		0.39	0.41

Mb, megabase; ASP, affected sib-pair analysis. The two-point lod scores are based on the analysis of individuals with MA.