Consistently Replicating Locus Linked to Migraine on 10q22-q23

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Here, we present the results of two genome-wide scans in two diverse populations in which a consistent use of recently introduced migraine-phenotyping methods detects and replicates a locus on 10q22-q23, with an additional independent replication. No genetic variants have been convincingly established in migraine, and although several loci have been reported, none of them has been consistently replicated. We employed the three known migraine-phenotyping methods (clinical end diagnosis, latent-class analysis, and trait-component analysis) with robust multiple testing correction in a large sample set of 1675 individuals from 210 migraine families from Finland and Australia. Genome-wide multipoint linkage analysis that used the Kong and Cox exponential model in Finns detected a locus on 10q22-q23 with highly significant evidence of linkage (LOD 7.68 at 103 cM in female-specific analysis). The Australian sample showed a LOD score of 3.50 at the same locus (100 cM), as did the independent Finnish replication study (LOD score 2.41, at 102 cM). In addition, four previously reported loci on 8q21, 14q21, 18q12, and Xp21 were also replicated. A shared-segment analysis of 10q22-q23 linked Finnish families identified a 1.6-9.5 cM segment, centered on 101 cM, which shows in-family homology in 95% of affected Finns. This region was further studied with 1323 SNPs. Although no significant association was observed, four regions warranting follow-up studies were identified. These results support the use of symptomology-based phenotyping in migraine and suggest that the 10q22-q23 locus probably contains one or more migraine susceptibility variants.

Introduction

Migraine (MIM 157300) is the most common cause of chronic episodic severe headache. It affects some 15% of the adult population and has a well-established genetic component[1–4] on the basis of family and twin studies. It is more prevalent among women, with a ratio of roughly one male to every three female migraineurs.[1] Migraine is the most common neurological cause of a doctor visit and places a heavy financial, social, and psychological burden on a significant part of the general population. The estimated annual cost of migraine in Europe is €27 billion.[5]

Although evidence from family studies and twin studies have demonstrated the contribution of genetic factors to migraine susceptibility,[3,6,7] identification of specific genetic variants for common forms of migraine has not been forthcoming. No variants predisposing to common forms of migraine have been convincingly established, and no whole-genome association (WGA) studies have been reported for any headache disorders to date. Genome-wide linkage studies have pointed to several loci in both migraine with and without aura.[8–15] Unfortunately, so far there has been little concordance between linkage reports because most studies have identified a locus or two, which have not been convincingly replicated in other studies. Applying findings from other complex disorders suggests that the lack of progress in gene identification may be attributable to etiologic or phenotypic heterogeneity, gene-environment interaction, or epistasis. Another possible reason is genetic (locus) heterogeneity, in which only a subset of pedigrees segregates markers linked to a particular risk locus. Then, even if the study sample consists of a large number of families, individual large families within the sample carrying rare, relatively high-impact gene variations predisposing to migraine can be overly represented in the linkage signal. This would explain some of the difficulties with replication, and better understanding of how to account for these factors would help in targeting future studies as well as help in interpreting results from whole-genome association studies. Finally, we hypothesize that one of the reasons behind this inconsistency might be related to the difficulty of phenotyping headache disorders, causing heterogeneity in sample ascertainment.

One of the major impediments to gene identification of migraine is the lack of valid biological markers with which

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Table 1. Diagnostic Criteria for Migraine without Aura and for the Headache Associated with Typical Aura with Migraine Headache According to the International Classification of Headache Disorders, Second Edition

1.1. Migraine without Aura[a]
A. At least five attacks fulfilling criteria B–D
B. Headache attacks lasting 4–72 hr (untreated or unsuccessfully treated)
C. Headache has at least two of the following characteristics:
1.Unilateral location
2.Pulsating quality
3.Moderate or severe intensity (inhibits or prohibits daily activities)
4.Aggravation by walking stairs or similar routine physical activity
D. During headache, at least one of the following:
1.Nausea and / or vomiting
2.Photophobia and phonophobia
E. [Exclusion of secondary causes of headache]

[a] For typical aura with migraine headache (1.2.1): "Headache fulfilling criteria B–D for 1.1 Migraine without aura begins during the aura or follows aura within 60 min."

a presumptive diagnosis of migraine can be made. A migraine diagnosis is based on fulfillment of symptom criteria formulated by the International Headache Society (IHS).[16,17] The criteria define two main subtypes of migraine, migraine with aura (MA) and migraine without aura (MO), which together account for a majority of all migraine. Most studies performed so far have used the migraine end diagnosis as the primary phenotype, i.e., by considering only patients with either MA or MO diagnosis as affected in analysis. Although the IHS classification works well and is fundamental in clinical practice, it may not be an optimal strategy for uncovering underlying genetic mechanisms and pathways contributing to the disease. The second edition of the IHS classification[17] introduced the same basic symptom criteria (see Table 1) for MO and typical aura with migraine headache (a major subgroup of MA). This, combined with studies suggesting migraine with and without aura are manifestations of the same underlying disorder,[18,19] have led to joint genetic analysis of patients from both diagnosis groups. This, in turn, gave rise to the idea of concentrating on one or few cardinal migraine symptoms, which might better reflect the underlying pathophysiology.

Two alternative analytic strategies, one utilizing latent classes,[18] the other examining trait components,[11] have recently been developed for use in genetic studies of migraine. In the latent-class analysis (LCA) approach, individuals are classified into empirically derived groups on the basis of patterns of IHS symptom clustering observed in a large Australian twin sample.[18] Although considerably more individuals were classified as being affected with "migrainous headache" via LCA (prevalence 36% versus 15% for clinically determined migraine), additional studies in Australian[18] and Dutch[20] twin populations have shown that the LCA classification is able to demonstrate linkage to loci undetectable with only the end diagnosis. An alternate strategy is the trait-component analysis (TCA) approach, which takes direct advantage of the available clinical information in the IHS symptom data in order to classify the patients into groups. This approach has the advantage of reflecting known variables obtained directly from patients with no intervening hypotheses about latent structure and relationships of the traits. It is also simple to implement from patient questionnaires or interviews and has proved to be successful in demonstrating linkage to loci undetectable with traditional methods in a previous Finnish study.[11] Encouraged by our previous results with these alternative phenotyping strategies and their potential to facilitate data integration from different phenotyping schemes, we genotyped and analyzed two new, independent genome-wide linkage scans from Finland and Australia. The samples are of roughly equal size but have differences in their ascertainment strategies and pedigree structures, allowing us to test the phenotyping methods in a variety of conditions. Further, the special population history of Finns provides an advantage to potential restriction of any linked locus through extended haplotype sharing.

Material and Methods

Patients

The Finnish study sample for the genome-wide scan consisted of 690 migraine patients and their relatives (407 women and 283 men) in 58 independent, multigenerational families. The Australian sample consisted of 661 individuals (420 women and 241 men) in 125 independent nuclear families. The Finnish replication sample consisted of 324 migraine patients (202 women and 122 men) in 27 independent, multigenerational families. In total, we studied 1675 individuals from 210 independent families. All participants gave informed consent, and approval to conduct the research was obtained from the Helsinki University Central Hospital Ethics Committee for the Finnish study and from the Queensland Institute of Medical Research (QIMR) Human Research Ethics Committee and the Australian Twin Registry for the Australian study. For the follow-up association study, two study samples from the Finnish population were used. The first study sample consisted of 39 unrelated trios with discordant parents selected so that both the affected parent and an affected offspring carry the family-specific segregating "risk haplotype" and that the unaffected parent did not. The case-control set contained 256 unrelated MA cases selected from the Finnish patient collection and 230 controls from a Helsinki-based-population control sample.

Diagnoses and Phenotypes

The Finnish families were selected from a large Finnish migraine patient collection, ascertained from neurology clinics nation wide during the last 15 years. The patients have been collected from families with three or more affected members fulfilling migraine criteria upon admission. Data on IHS attack symptoms as well as other clinical features were collected with the validated Finnish Migraine Specific Questionnaire for Family Studies ($FMSQ_{FS}$)[21] and by a neurologist's examination of index patients. The same neurologist (M.K.) diagnosed all Finnish patients. The replication sample consists of large families selected from the same patient collection, with a preference for more severe migraine patients, including those with hemiparesis symptoms, because of findings in the Finnish genome-wide sample.

Table 2. Distribution of Migraine Diagnoses within the Finnish Study Samples

	Finns				Australians	
	Genome-wide Sample		Replication Sample		Genome-wide Sample	
Diagnosis	n	of Total	n	of Total	n	of Total
Pure MA[a]	169	24%	44	14%	191	24%
Pure MO	79	11%	35	11%	78	10%
Unclassified MA[b]	89	13%	33	10%		
Mixed migraine[c]	110	16%	78	24%		
Equivalent migraine	7	1%	2	1%		
Headache	26	4%	11	3%		
No headache	169	24%	61	19%	230	28%
Possible migraine[d]	27	4%	18	6%		
Unknown	19	3%	42	13%	305	38%
MA end diagnosis	368	53%	155	48%	191	24%
Total	690	100%	324	100%	804	100%

Note the Australian symptom data do not allow the strict separation of migraine with aura patients into unclassified MA, mixed migraine, and pure migraine with aura subgroups.

[a] Pure MA refers to patients with all attacks fulfilling IHS criteria for migraine with aura.

[b] Unclassified MA refers to an additional, non-IHS diagnosis group for patients that cannot be grouped into any of the defined IHS categories. Patients in this category suffer from attacks in which clearly aural features are present but not in a form recognized by the current diagnostic criteria.

[c] Mixed migraine refers to a patient group in which attacks both with and without aura are commonly present.

[d] Possible migraine refers to a patient group with episodic headache with some migrainous features, who may or may not fulfill one of the probable migraine (1.6) diagnoses of the IHS criteria but miss required aspects of migraine with or without aura.

The Australian families were selected from two population-based twin cohorts, one of nuclear families of twins born between 1902 and 1964[22] and one of twins born between 1964 and 1971,[23] with an overall prevalence of 15.3% of IHS migraine without aura. The included pedigrees were selected on the basis of having at least one pair of siblings affected for the common LCA-derived "migrainous headache" phenotype (prevalence of 36%[18]) and then prioritized on the maximum number of available siblings, irrespective of affection status. Data on IHS attack symptoms[16,17] were gathered with an extensive semistructured telephone interview that included diagnostic questions for migraine (Australian questionnaires for the older and younger cohorts, see Web Resources), developed by an experienced migraine researcher (K.R.M.).[24] Using a similar screening approach, Stewart et al.[25] obtained a 92.6% positive predictive value of their telephone interview diagnosis compared with their clinical examination. For the younger cohort, data for two IHS diagnostic variables (Table 1), nausea and vomiting (ICHD-II code: 1.1.D.1), were recorded together. For the older cohort, data on three variables, pain intensity (1.1.C.3), typical attack length (1.1.B), and whether patients have had at least five attacks during lifetime (1.1.A), were unavailable, but symptom patterns of the younger cohort were used to extrapolate those phenotypes for the older cohort. Data on whether an individual's headache was aggravated by walking stairs or similar routine physical activity (1.1.C.4) were missing for both cohorts, and thus that trait was excluded from the study. We used an answer to a visual aura-specific question to determine the MA end diagnosis.

Table 3. Number of Affecteds, Frequencies of Individual Trait Components, and the Gender Proportions of Those Affected for All Traits and Trait Groups within the Study Samples

Phenotype (n)	of Total	Finns	Australians	Males	Females
Total subjects (1675)	-	61%	39%	39%	61%
MA end diagnosis (621)	37%	41%	31%	21%	49%
Latent class CL23[a] (790)	47%	48%	45%	29%	60%
Latent class CL3 (599)	36%	36%	36%	15%	49%
Attack length (781)	47%	45%	49%	35%	60%
Unilaterality (727)	43%	48%	36%	30%	52%
Pulsation (778)	46%	49%	42%	35%	54%
Intensity (1033)	62%	67%	53%	46%	71%
Nausea/vomiting (870)	52%	55%	48%	34%	63%
Photophobia (918)	55%	59%	47%	37%	66%
Phonophobia (826)	49%	50%	47%	31%	61%

[a] Refers to a combination of latent classes CL2 and CL3.

Three different phenotype groups were prepared. "MA end diagnosis" covers all migraine with aura patients and includes individuals from diagnosis groups "pure MA," "unclassified MA," and "mixed migraine" as affected (see Table 2 for definitions). Table 2 details the diagnosis distribution within the study samples, including a detailed diagnosis breakdown for the two Finnish study samples, in which the larger amount of available clinical information and expertise allows for a higher diagnostic specificity for the clinical diagnosis. The Australian study questionnaire has fewer migraine-specific questions and is designed to identify migraine with high sensitivity but does not allow for distinguishing between different subtypes of MA. The latent-class definitions were estimated from each patients' symptom distribution with the same algorithm as in the original LCA study.[18] In brief, of the four latent cluster groups in LCA (termed CL0, CL1, CL2, and CL3), all individuals satisfying the IHS MA or MO diagnostic criteria are encompassed by groups CL2 and CL3, and the combination of these two groups will be referred to as "LCA migrainous headache." Group CL3, which has the majority of MA patients, is referred to as "LCA severe migraine." Trait-component phenotypes were recorded directly from the questionnaire data of all patients fulfilling any migraine diagnosis. Table 3 summarizes the proportions of the different phenotypes.

Genotyping

All genotyping was performed in the Finnish Genome Center, with the same equipment and conditions. The genotyping procedure was conducted with standard methods on the ABI or the MegaBACE genotyping systems. Genotyping was based on the LMS-MD10 microsatellite marker set (Applied Biosystems, Foster City, CA, USA). The marker set uses 387 markers for a 9.5 cM average intermarker distance and covered all autosomes and the X chromosome. For the ABI system, genotyping was performed with the ABI 3730 capillary sequencing instrument, and PCR products were resolved with the ABI 3730 data collection software and sized with the Genemapper software package from Applied Biosystems. For the MegaBACE system, capillary electrophoresis employed by the MegaBACE 1000 DNA Sequencing System (GE Healthcare Bio-Sciences, Piscataway, NJ, USA), was used for separating DNA fragments. Alleles for this system were called by the MegaBACE Genetic Profiler 1.5 software. In addition, seven more markers were genotyped at chromosome 10q22-q23, resulting in a coverage of 2.21 cM average intermarker distance from marker

D10S218 to D10S2470. The Finnish replication sample was genotyped only for these markers. All genotypes were verified by human inspection, and the PedCheck1.1[26] computer program was used for detecting genotyping errors.

For the follow-up association study, an Illumina Golden Gate assay (Illumina, San Diego, CA, USA) was used to genotype 1536 single-nucleotide polymorphisms (SNPs) in altogether 564 individuals across the region defined by the shared haplotype (chr10, 78.233–88.884 Mb, NCBI build 35) at the Broad Institute. These 1536 SNPs on chromosome 10 (build 35, 78.233−88.884 Mb) were selected as tag-SNPs with Haploview's Tagger-option with CEU population in the HapMap SNP set (v21), and we selected to tag SNPs with minor allele frequency $\geq$10% and r2 threshold of $\geq$0.8. The selected 1536 tag SNPs tagged 94% of the 8290 SNPs (MAF $\geq$0.10) with r2 $\geq$0.8 and 99% of the SNPs with r2 $\geq$0.5. The Illumina BeadStudio software version 3.1.0.0 (Illumina) was used for calling the SNP genotypes, and each SNP was evaluated for quality of the genotypes. Only samples that had success rate of $\geq$97% and SNPs with 95% were considered in the statistical analyses of the SNP data, and thus of the 1536 original SNPs, 1323 passed our rigorous quality control. Because of the difficulty involved in genotyping the region around the known CNV at ~81.3 Mb, there were no successfully genotyped SNPs between 81,058,202 and 81,674,055 base pairs, resulting in a 615 kilobase gap in the assay coverage.

Linkage and Association Analysis

For the genome-wide analyses, multipoint nonparametric linkage analysis was performed with the MERLIN computer program.[27] The MERLIN NPL_{pairs} and NPL_{qtl} Z score statistics are implemented in the general framework of Whittemore and Halpern.[28] These Z scores are used by MERLIN to construct a likelihood ratio test for linkage and define a LOD score statistic with the exponential modeling procedure of Kong and Cox.[29]

For the Finnish families, in line with our previous research,[8,11] we employed an affecteds-only strategy (i.e., all individuals not classified as affected were considered to have an "unknown" phenotype) to allow for reduced penetrance, lack of environmental exposure, etc. We used the nonparametric MERLIN NPL_{pairs} Z score statistic[30] to test for increased allele sharing among affected individuals. To avoid biasing our results on possible overrepresented rare variants in a few large families, we also analyzed the Finnish genome-wide sample as nuclear families. For consistency with the previous Australian genome-wide linkage scan,[8] in order to use the information from unaffected individuals, we used a nonparametric quantitative trait linkage (NPL_{qtl} Z-score) statistic for the analyses of the Australian families in order to obtain additional linkage information from unaffected individuals. In this analysis, affected individuals were coded as "1," unaffected individuals were coded as "0," and those with missing phenotypes were coded as "x." The validity of this, as well as the original regression Haseman-Elston approach[31] for binary traits, has been proven consistently.[32] For the combined genome-wide analysis of Finnish and Australian pedigrees, we used nuclear families to avoid biasing the signal because of the larger Finnish families, and the NPL_{pairs} Z-score statistic was used with the usual "affection" phenotype coding of 0, 1, and 2 to represent unknown/missing, unaffected, and affected individuals, respectively. In addition, we performed a sex-specific analysis by alternatively considering only the affected females or males as "true" affecteds and treating the affecteds of the other gender as having an "unknown" phenotype. In addition, a haplotype shared-segment analysis was performed in the Finnish families. The GENEHUNTER software,[33] version 2.1_r5beta, was used for construction of pedigrees showing the paternal and maternal haplotypes for the additional markers at this locus for the families showing a family-specific NPL_{all} score greater than 1.00 at the location of the highest LOD score.

For the follow-up association study, PLINK software version 1.00[34] was used for all analyses. We employed the DFAM analysis (−dfam) to detect association in the combined set of trios and the case-control subjects. Results were corrected through adaptive permutation (−perm) with PLINK default settings.

Significance Limits

To account for all the phenotypes tested, we needed to apply robust correction for multiple testing. To start, rather than to use the significance thresholds of Lander-Kruglyak (L-K),[35] conservative for microsatellite-based linkage scans due to the unrealistic assumption of having complete (100%) inheritance information, we estimated the significance thresholds for affected sibpair analysis of 400 markers by using the formulae presented by Feingold et al.[36] The L-K threshold for significant evidence of linkage ($p = 0.000022$, corresponding to a standard LOD score of 3.63) is decreased to $p = 0.00009$ (corresponding to a LOD score of 3.05). Similarly, the threshold for suggestive linkage is reduced from $p = 0.00074$ (LOD score of 2.19) to $p = 0.0023$ (LOD score of 1.74).[37] These theoretically derived thresholds are consistent with those obtained via simulation by ourselves[8] and others.[38–41] To correct for the multiple phenotypes (including the sex-specific analyses) used in this study, we applied the program matSpD (see Web Resources) to estimate the equivalent total number of independent tests performed (six), resulting in robust Bonferroni-corrected significance thresholds of 6.18 [$5.40 + \log_{10}(6)$] for highly significant evidence of linkage, 3.83 [$3.05 + \log_{10}(6)$] for significant evidence of linkage, and 2.52 [$1.74 + \log_{10}(6)$] for suggestive evidence. For the replication set, we applied the L-K replication threshold of LOD 1.8 (nominal evidence of linkage, $p = 0.01$, for five independent tests), equal to fine mapping a 10 cM area.[35] For the follow-up association study, we used the snpSpD program (see Web Resources) to estimate the number of independent SNP tests after accounting for LD (761.7), resulting in Bonferroni-corrected significance threshold of 6.73×10^{-5}.

Results

Genome-wide multipoint linkage analysis of 387 microsatellite markers was performed in two independent study samples; this was followed by an analysis of a locus-specific Finnish replication sample. All samples were analyzed separately as well as jointly. A locus on 10q22-q23 showed significant evidence of linkage in Finns as well as in the joint analysis and suggestive evidence of linkage in the Australian study. A sex-specific analysis, considering only females as affected, improved the linkage signal to the level of highly significant evidence of linkage. No other loci showed linkage in both samples. Population-specific loci on 2p12, 8q12, and Xp22 showed suggestive evidence of linkage.

Genome-wide Population-Specific Linkage Analysis

We first wanted to identify regions linked to any of the migraine traits in the individual study populations. In

Table 4. Phenotypes Showing Genome-wide Significant LOD Scores at the 10q22-q23 Locus and Their LOD Scores in Each Sample

Phenotype	Finnish, NPL_{pairs}, 103 cM	Australian, NPL_{qtl}, 106 cM	Joint, NPL_{pairs}, 102 cM
MA end diagnosis	*4.65*	0.00	1.58
LCA migrainous headache	*4.81*	0.91	3.00
TCA unilaterality	*5.18*	0.00	0.62
TCA pulsation	*4.24*	3.50	*4.62*
TCA pain intensity	*5.03*	1.32	*3.75*
TCA nausea/vomiting	*3.90*	0.25	2.88
TCA photophobia	*4.22*	0.11	2.40
TCA phonophobia	*5.03*	0.00	1.63

Note that numbers in italics represent genome-wide significant evidence of linkage (LOD $>$ 3.83).

the Finnish study sample, the MERLIN NPL_{pairs} analysis showed significant evidence of linkage to a locus on 10q22-q23. The highest LOD score (5.50) was observed at 103 cM with the TCA unilaterality phenotype, with the 95% CI placing the locus between 99 cM and 114 cM. Significant evidence of linkage at this locus was also shown by the MA end diagnosis, LCA migrainous headache, and five additional TCA phenotypes (see Table 4). In the Finnish study sample, no other chromosomal region showed significant evidence of linkage, and only two regions showed suggestive evidence of linkage (on 2p12, NPL_{pairs} LOD score 2.60 at 100 cM with TCA pulsation phenotype; 1.93 for MA end diagnosis, and 1.74 for LCA migraine and on Xp22, NPL_{pairs} LOD score 2.96 at 39 cM with TCA pulsation phenotype, 1.19 for MA end diagnosis, and 1.72 for LCA severe migraine), although a previously detected locus on 18q12[9–12] showed sufficient evidence for replication (NPL_{pairs} LOD 2.46 at 86 cM with TCA attack-length phenotype, 0.21 for MA end diagnosis, and 0.41 for LCA migrainous headache). Encouragingly, the 10q22-q23 locus is robustly replicated in the Australian study sample with a highly suggestive NPL_{qtl} score of 3.50 at 100 cM with the TCA pulsation trait, with the 95% CI located between 94 cM and 115 cM. Other phenotyping methods provided modest signals in the Australian study sample at the 10q22-q23 locus. In the Australian sample, suggestive evidence of linkage was found to a region on 8q12 (NPL_{qtl} LOD of 2.63 at 86 cM with the TCA pain intensity phenotype, 0.29 for MA end diagnosis, and 1.27 for LCA migrainous headache), and a previously detected locus on 14q21 was replicated (NPL_{qtl} LOD 2.23 at 26 cM with TCA pain intensity phenotype, 0.24 for MA end diagnosis, and 1.68 for LCA migrainous headache). The genome-wide results for all traits are shown in Figure 1.

Genome-wide Joint Analysis of Australian and Finnish Study Samples

Results of a joint MERLIN NPL_{pairs} analysis yielded significant evidence of linkage to the same region as in the

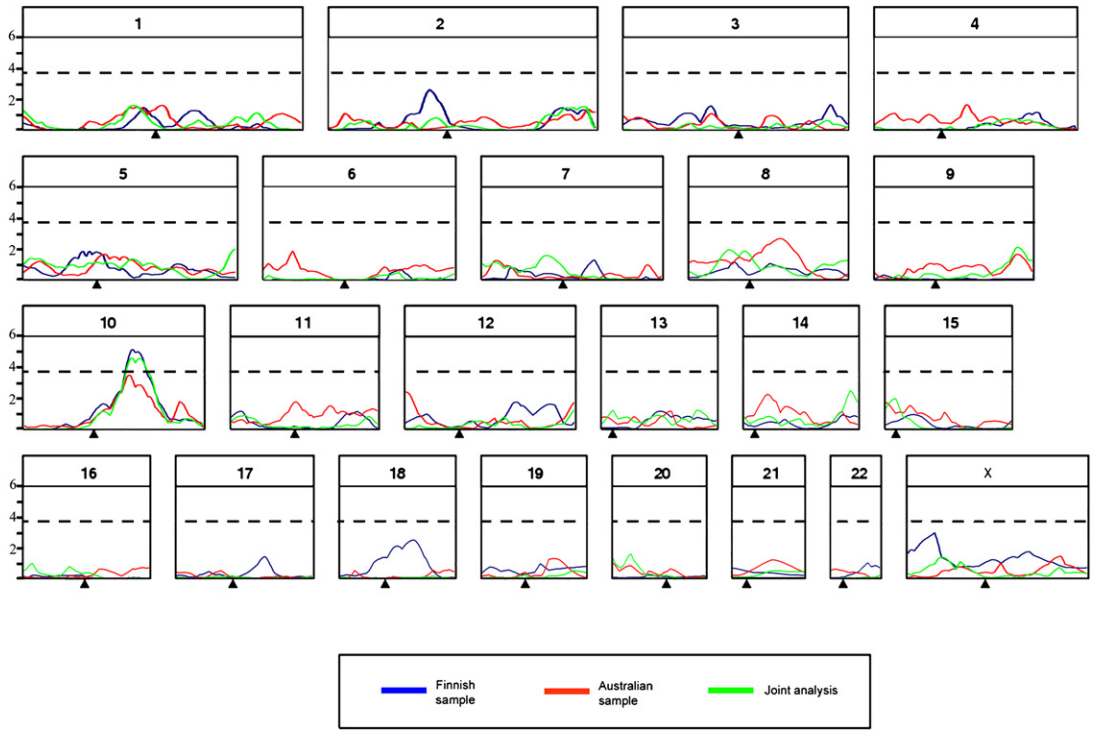

Figure 1. Maximum LOD Scores in the Genome-wide Screen

The graphs show values across all phenotypes and phenotyping methods for the Finnish study sample in the NPL_{pairs} analysis, the Australian study sample in the NPL_{qtl} analysis, and the NPL_{pairs} analysis performed on both study samples together. The dotted line denotes the level of significant evidence of linkage (LOD $>$ 3.83).

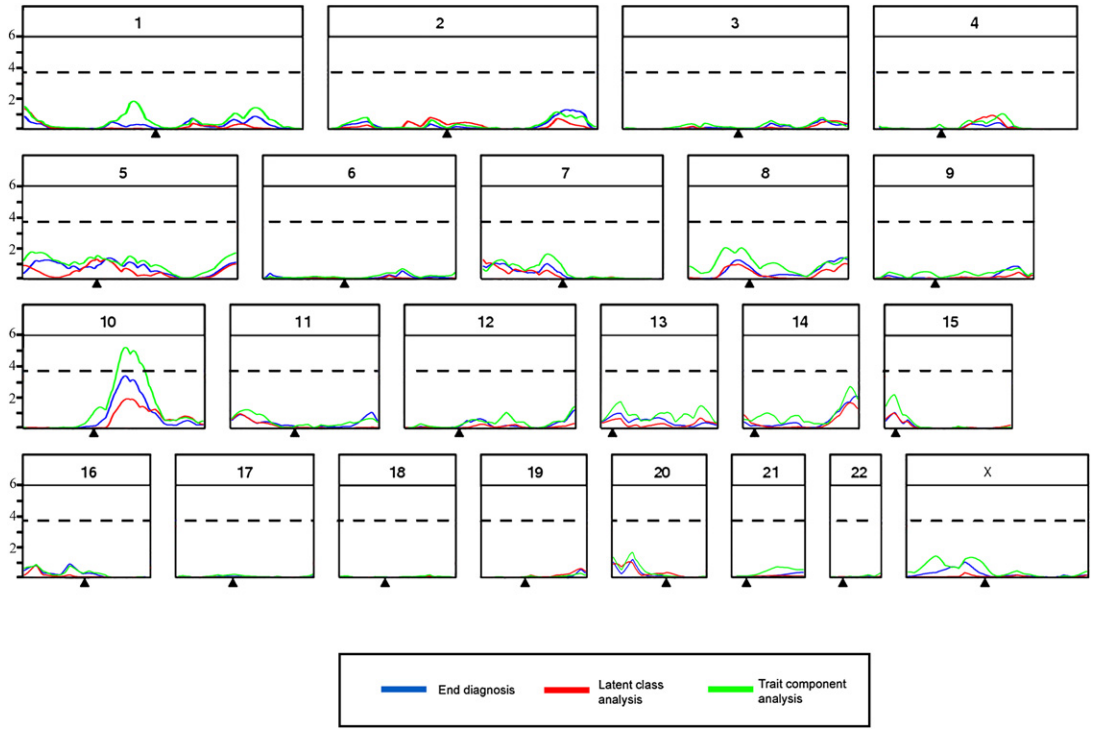

Figure 2. Genome-wide Comparison of the Three Genotyping Methods in the Combined Study Sample
The graphs show the highest LOD score detected with each phenotyping method in the joint analysis of the two genome-wide screens in the NPL_{pairs} analysis performed with both study samples together. The horizontal lines and boxes indicate the maximum LOD scores at 10q22-q23 for each method. The dotted line denotes the level of significant evidence of linkage (LOD $>$ 3.83).

individual analysis, between 98 cM and 117 cM at 10q22-q23. The highest LOD score (4.62) was found at 102 cM with the TCA pulsation phenotype, and significant evidence of linkage was also detected with TCA pain-intensity phenotypes (see Table 4). Neither of the regions showing suggestive linkage in only one sample showed evidence of linkage above nominal level (2p12 highest NPL_{pairs} LOD score 0.57, for 8q12, 1.01; and for Xp22, 1.36) in the joint analysis. Comparison of results from each of the three phenotyping methods in the joint analysis is presented in Figure 2.

Fine Mapping the Locus on 10q22-q23

Seven additional markers were genotyped in both initial study samples to increase the available linkage information across the implicated 10q22-q23 region. When including those markers in the joint-linkage analysis, the highest peak was found at 106 cM (NPL_{pairs} LOD score of 4.11 with the TCA pulsation phenotype, 1.28 for MA end diagnosis, and 2.16 for LCA migrainous headache). These results are detailed in Figure 3.

Finnish Replication Study

We genotyped an independent Finnish replication sample of 27 families for the seven additional microsatellite markers at the 10q22-q23 locus to further strengthen the evidence of linkage. Because the families providing most of the linkage signals to the 10q22-q23 locus in the genome-wide study were found to suffer from a severe form of migraine that included some hemiparesis symptoms (although not severe enough to qualify as familial or sporadic hemiplegic migraine), this clinical phenotype was used as the basis of selecting the families for the replication study (see Table 5). In the linkage analysis, the highest peak was found at 102 cM with the TCA pulsation

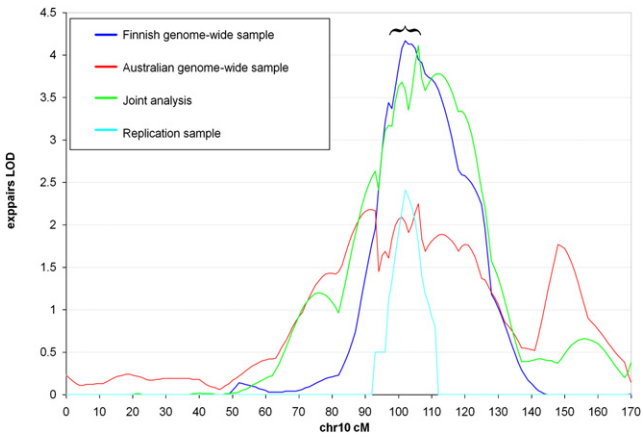

Figure 3. Positioning the Linkage Peaks on Chromosome 10
The graphs show maximum attained LOD scores in each study sample in the Merlin multipoint analyses, including the seven additional microsatellite markers. The bracket denotes the area covered by the family-specific haplotype segregating with the affection status.

Table 5. The Number and Proportion of Migraineurs with Hemiparesis and Hemisensory Symptoms in the Finnish Study Samples

	Previous Genome-wide Sample[11]	Current Genome-wide Sample	Replication Sample
n in total sample	441 (-)	690 (-)	324 (-)
with hemiparesis symptoms	42 (9.5%)	67 (9.7%)	51 (15.7%)
with hemisensory symptoms	87 (19.7%)	117 (17.0%)	70 (21.6%)

phenotype (NPL_{pairs} LOD score of 2.41), sufficient for replication (LOD > 1.8).

Sex-Specific Findings

In line with previous studies[11,12] that have suggested sex-specific effects at linked loci, we performed a sex-specific analysis for chromosome 10. In the Australian study, sex-specific analyses yielded no improvement in the linkage signal. However, in the Finnish study sample, considering only affected females yielded a considerable increase in the LOD score, resulting in highly significant evidence of linkage with a number of phenotypes, including the MA end diagnosis. The highest NPL_{pairs} LOD score was 7.68 (at 103 cM, TCA phonophobia phenotype, 4.37 for MA end diagnosis, and 5.33 for LCA migrainous headache). In contrast, considering only affected males, the linkage signal was below the level of nominal evidence of linkage (highest NPL_{pairs} LOD score 0.20 at the same location). For the Finnish females, all of the studied phenotypes except LCA severe migraine showed significant evidence of linkage. For the joint analysis, considering only females and nuclear families produced a significant LOD score of 4.11 (at 106 cM with the TCA photophobia phenotype, 2.52 for MA end diagnosis, and 3.19 for LCA migrainous headache). Female-specific results are detailed in Figure 4.

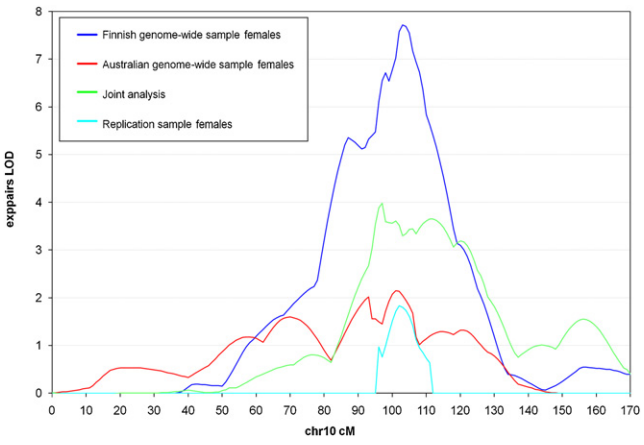

Figure 4. Female-Specific Multipoint Linkage Results on Chromosome 10

The graphs show the results obtained with the TCA pulsation phenotype, which gives the highest evidence of linkage in each sample.

Pedigree Identifier	D10S										
	1652	537	535	109	1730	605	569	1786	1686	1687	185
18	n/a	5	5	3	n/a	6	8	5	8	2	n/a
43	n	n	6	4	n/a	2	1	8	1	3	n/a
85	n	9	5	3	8	5	7	7	7	2	5
119	n	n	7	2	9	2	1	6	1	3	n/a
126	n	n	6	2	4	4	8	3	5	n	n
127	n	n	n	n	n	n	n	3	8	1	2
151	n	n	n	3	4	2	8	3	7	1	2
158	n	n	2	5	8	5	9	7	6	3	5
189	9	9	4	2	7	1	8	1	3	6	5
203	n	n	n	n	n	n	8	5	8	2	5
221	n	n	2	4	5	2	5	7	1	5	2
230	n	2	5	7	4	2	5	2	6	3	5
257	9	6	3	2	7	2	5	3	2	1	5
291	n	n	n	n	n	n	n	2	2	1	2
523	n	n	n	n	n	n	8	3	8	8	5
573	n	n	n	1	n/a	2	5	2	2	1	5
732	n/a	n	6	3	4	5	1	5	1	5	n/a
736	n/a	8	6	2	9	4	1	6	8	4	n/a
778	n/a	n	n	n	n	5	1	5	7	4	n/a
19703	n	4	5	2	7	2	8	7	8	5	10
23901	9	4	5	7	4	2	3	7	5	2	n
				KCNMA1				*NRG3*		*GRID1*	

Figure 5. Haplotype Distribution among the Finnish Families with Family-Specific LOD Scores over 1.00 at the Location of the Highest Linkage Signal in Finns, 103 cM

This figure shows the family-specific haplotype segregating with the affection status on chromosome 10q22-q23, for the roughly 30 Mb area spanning the markers D10S1652 and D10S185 around the linkage peak. The lightly shaded area represents the haplotype block shared by affected members of the family, and the darker shading indicates the region shared by all affected family members across families. "N/a" denotes an unavailable genotype, and "n" denotes multiple different alleles in affected family members. The bottom of the figure shows the largest transcribed genes in the region, in scale relative to the distances between the microsatellites.

Haplotype Analysis at 10q22-q23

Because the Finnish study sample is from a population with a limited number of founders and multiple bottlenecks in the population history, we performed a haplotype shared-segment analysis to further restrict the linked region and identify the most probable location of the disease predisposing variants. The analysis was conducted in those 21 Finnish families that had a family-specific nonparametric linkage (NPL) score greater than 1.00 (as measured by GENEHUNTER) at the location of the highest linkage signal (97.5 cM), both from the genome-wide sample and the replication sample. These families contain 178 individuals, of which 99 (or 56%) are considered affected according to our clinical MA end diagnosis. Ninety-five percent (94 out of 99 subjects) shared the family-specific haplotype between markers D10S1786 (103.3 cM) and D10S1686 (104.9 cM). Considering the locations of the flanking markers (D10S569 at 97.5 cM and D10S1687 at 107.3 cM), the detected haplotype is between 1.6 and 9.8 cM wide (1.6 – 9.6 Mb). Restricting the area to this region

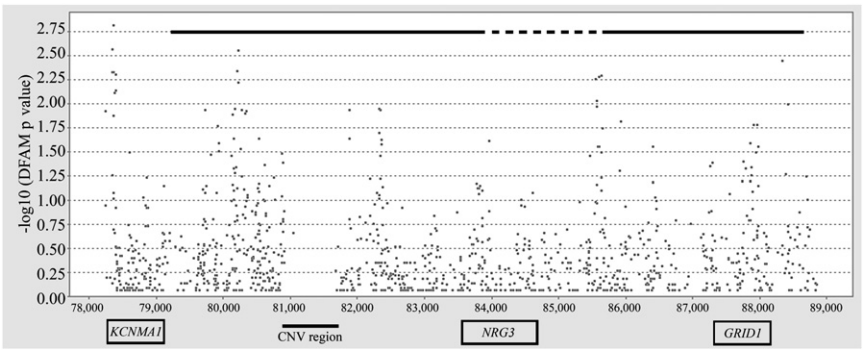

Figure 6. Results of the Follow-Up Association Study that Used 39 Trios, 256 Unrelated Cases, and 230 Controls in the DFAM Association Analysis

The dotted line at the top of the figure denotes the minimum known length of the family-specific segregating microsatellite haplotype (see Figure 6), and the solid line denotes its maximum possible length. The bottom of the figure shows the largest transcribed genes in the region, in scale according to the isoform with the largest genomic size, as well as the area of the known CNV.

was accomplished on the basis of three and four informative recombinations, respectively. Results of this analysis are detailed in Figure 5.

Follow-up Association Study

The trio and case-control samples were analyzed with the DFAM analysis option of PLINK,[34] which allows for the combination of trio and case-control data. None of the SNPs showed association exceeding the significance threshold of 6.73×10^{-5}. The highest association was detected with SNP rs1873695 (p value 9.22×10^{-4}; *KCNMA1* intronic), with several adjacent SNPs showing a similar level of association (rs2131218: 0.0035, rs16934025: 0.0019). Three other regions show association scores under the 0.005 level with several adjacent markers: rs10458664 (0.0035, outside any known gene), rs7906586 (p = value 0.0025, outside any known gene), and rs2691052 (0.002, outside any known gene) (see Figure 6 for results).

Discussion

In the present study, we detected highly significant evidence of linkage to 10q22-q23 and replicated the finding in two diverse populations. The locus was detected with all three phenotyping methods used, which alternatively concentrate on the presence of aura, IHS symptom clustering, or the individual migraine symptoms. The consistency of linkage findings across studies with different ascertainment schemes and phenotyping methods provides compelling evidence for the strength of this finding.

As is often the case in complex traits, the linkage peaks defining the detected 10q22-q23 region are relatively broad (between 8 cM and 21 cM wide, depending on the method of analysis), and the number of susceptibility loci within this region cannot be predicted. However, given that the analysis peaks all converge on a narrow (under 5 cM) area, which contains the area defined by the shared-segment analysis, there is therefore strong evidence for constraining the peak between 97.5 cM and 104.9 cM (see Figure 3). This interval contains two obvious functional candidate genes. *KCNMA1* is a Maxi-K, calcium-level detecting potassium channel, which is involved in ion transport in a similar manner to the three known genes involved in the molecular pathology of familial hemiplegic migraine (see Table S1A), a related Mendelian disorder (*CACNA1A*, *ATP1A2*, and *SCN1A*, MIM 141500, MIM 602481, and MIM 609634, respectively). *NRG3*, located directly in the middle of the narrowest peak, is a gene belonging to the neuregulin family of growth and differentiation factors that are related to epidermal growth factor, which plays a role in oligodendrocyte survival.

Overall, there is an encouraging consistency between the results of this study and the previous Finnish[11] and Australian[8] studies. An overview of current and previous results at 10q22-q23 can be seen in Figure 7. The three other chromosomal areas showing evidence of significant or suggestive linkage to migraine in more than one report and in more than one population prior to this study are on chromosomes 4q21-q31, 15q11-q13, and 18q12. The previously reported chromosome 4q locus seems to be exceptionally broad; whereas the linkage in the Icelandic population is reported at 4q21,[12] previous Finnish studies identified two peaks at 4q24[9] and at 4q28-q31,[11] making the total linked region up 50 cM wide. In this study, we

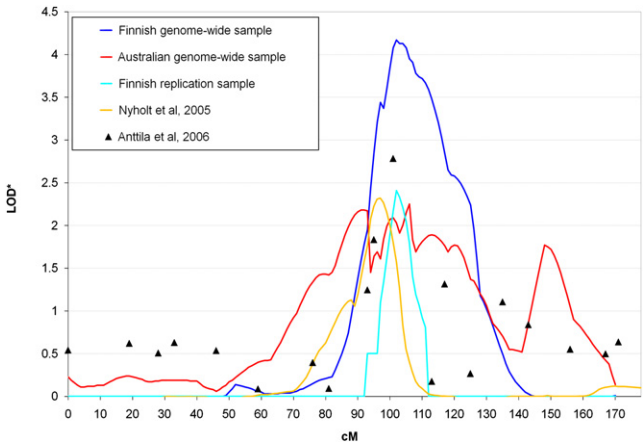

Figure 7. Previously Reported Migraine Linkage Results at the 10q22-q23 Locus Plotted Together with Results of This Study

were unable to replicate this locus, although closer examination of the family-specific results reveals the existence of a subset of families (approximately one-fourth of the total sample in both Australians and Finns) with family-specific Z scores up to 2.4, even though the overall evidence of linkage was nominal. This was also the case with the previously reported 17p13 locus.[11] Similarly, the previously reported 15q11-q13 locus was undetectable in our study. The chromosome 18q12 locus has been detected in both the Icelandic[12] and Finnish samples[9,11] (although the Icelandic linkage was observed after a broader definition of migraine was applied and only females considered as affecteds), as well as in the previous Australian study.[10] This locus is also replicated here, with the same TCA attack-length phenotype as in the previous Finnish study, bringing the total number of study samples showing linkage to this locus to four. Finally, three loci previously linked to migraine in only one study sample are also replicated, two in their respective populations: the locus on 8q21 detected in Australians (NPL_{qtl} LOD score of 2.63) is within 10 cM from a previously detected Australian locus,[8] a previously reported locus on 14q21 detected in a large Italian family[14] is replicated in the Australian sample (NPL_{qtl} LOD score of 2.21 at 26 cM), and the peak on Xp22 (NPL_{pairs} LOD score of 3.05 in Finns) is only 7 cM from a locus on Xp21, detected in the previous Finnish study.[9,11] In total, of the seven loci reported in Finns so far (4q, 10q*, 12q, 15q, 17p, 18q*, and Xp*), this study replicates three (denoted by asterisks), as well as three of the five reported in Australians (5q, 8q*, 10q*, 13q, and 18p-18q*). Finally, given that only one (2p12) of the four loci with suggestive or higher evidence of linkage in this study (2p, 8q, 10q, and Xp) has not been reported previously, overall the extended phenotyping methods seem to facilitate replication, especially considering that only one locus (4q21-q24) has been replicated with end-diagnosis-based approaches. An overview of results at each OMIM-listed migraine loci can be seen in Table S1B.

A closer look into previous migraine scans showed that the 10q22-q23 locus had been implicated in our two scans[8,11] with suggestive evidence of linkage. The present and the two previous studies suggest that complementing the classical clinical migraine diagnosis with alternative phenotyping strategies can facilitate the identification of susceptibility-locus identification. Both the trait-component analysis and the latent-class analysis approaches have proven useful in this respect, although they have different premises and represent conceptual approaches. It is of interest to note that if only the MA end diagnosis had been used as the study phenotype, the 10q22-q23 locus would have been detected with suggestive or significant evidence of linkage in only one of the five recent migraine study samples in the Australian and Finnish populations (including the three reported here; corresponding success rates 2/5 for LCA, and 5/5 for TCA).[8,11] There are two likely explanations for the greater sensitivity of TCA and LCA over the clinical diagnosis: (1) LCA and especially TCA may better reflect underlying processes in migraine pathophysiology, and/or (2) these two methods can utilize the questionnaire-based information in a more optimal way to find informative individuals within the MA and MO patient pools, thus including more cases and informative meioses for the linkage analysis. Although advantages and disadvantages exist for any analytical approach, these and previous results suggest that the trait-component analysis may offer substantial gains over analysis of clinical (migraine with aura) or empirical (e.g., LCA) end diagnoses especially when the diagnostic information is incomplete. Given that both the end diagnosis and the latent classes are based on combining information from phenotype profiles, it is perhaps not surprising that they both lose more power compared to TCA when the amount of available information is less than complete. This could explain the differences in results between the phenotyping methods in the current Australian study sample. Furthermore, using individual trait components directly allows additional efforts to be concentrated on increasing the size of the study samples, without the need to collect progressively more and more detailed clinical diagnostic information to optimize the formulation of the end diagnosis. On the other hand, it is possible that the TCA findings are the result of detecting genes involved in the symptom-specific processes and not involved in the primary pathophysiology of migraine.

The role of pulsating pain trait is of particular interest. Repeatedly, pulsation seems to be the most sensitive individual trait for linkage-based locus identification, providing all of the highest results in the Australian sample and many of those in the Finnish sample. This is evident in the previous two other genome-wide scans as well; the best signal in the previous Australian study (5q21)[8] is predominantly driven by pulsation, as is the best locus in the previous Finnish study (17p13),[11] which showed significant evidence of linkage only with pulsation. In addition, it plays a major role in the 10q22-q23 results in this study. The reason for this remains speculative. One possible explanation is that pulsating pain is a symptom that is more easily recognized by patients and thus is more consistently recorded in interviews and questionnaires. This does not, however, exclude the possibility that pulsation is indeed the most characteristic symptom of a particular type of migraine and reflects some yet-unknown neurovascular mechanism and is thus associated with specific pathophysiological pathways. It should also be noted that pulsation or any other TCA trait was not used in the sample ascertainment; that is, the sample selection process is naive with respect to the traits. Another interesting finding is the role of hemiparesis symptoms; because the families contributing most to the 10q22-q23 locus suffered from a more severe form of migraine, they have a higher proportion of hemiplegic symptoms. After the families for the replication study were selected for a higher prevalence of this severe form, a similar effect was observed in the replication study, further underlining the contribution of this

clinical phenotype to the 10q22-q23 locus. Thus, we were able to extract a part of the clinical spectrum of migraine, concentrate on it in case selection, and predict and subsequently demonstrate linkage to a particular genetic locus with a small number of patients targeted for that particular aspect, which is both a novel and an encouraging finding. In addition, on the basis of the known ion-channel-centered molecular pathology of familial hemiplegic migraine,[42–44] this supports *KCNMA1* as a compelling candidate gene. Lastly, the difference between overall and sex-specific linkage results at 10q22-q23 seem to reflect a predominantly female-dominated inheritance pattern in the Finnish families linked to this locus; such a finding is somewhat to be expected because of the higher prevalence of migraine in women. However, this is not enough to explain the considerable increase in linkage signal when considering only females as affected. The same effect, though to a smaller degree, can also be seen in the Australian study sample, as well as in a previous Finnish[11] and an Icelandic study.[12] These results suggest that using gender as a covariate in future migraine studies might provide increase in power for the detection of new variants. Sex might also be an indicator for male-specific environmental or behavioral characteristics that hide the signal in men, perhaps related to the better ability of women to detect and elaborate on symptoms and signs in headache and migraine; whether this is related also to higher prevalence of migraine in women also needs to be examined.

Although no SNPs showed significant association in the follow-up study, four potentially interesting regions support additional studies. The highest association, although not high enough to be considered significant, was observed with one obvious candidate gene, *KCNMA1*. The three SNPs with the next highest scores were located outside known coding genes. However, given the high linkage signal in the region and the variance among the family-specific affected haplotypes, it is possible that this locus contains multiple susceptibility variants affecting migraine but that the sample used is too small to sufficiently discern between them. Thus, larger studies are warranted to see whether these findings can be replicated. The potential, suggestive association to *KCNMA1* is intriguing because the established FHM mutations are all located in proteins involved in ion transport.

A timely question is how linkage signals, as the chromosome 10q22-q23 locus reported here, correspond to association signals in WGA studies. Recent studies provide an opportunity to compare loci identified with these two different strategies that are based on different hypothesis. Although linkage studies are best suited to position relatively penetrant and possibly rare variants, WGA studies are designed to test the "common-diseases-common-variant" hypothesis. Although the number of WGA studies is still limited, some trends can be observed; there are examples of identification of both previously unidentified loci and confirmation of loci identified in linkage studies. Importantly, the WGA studies have identified new robustly replicated loci in regions not previously linked or associated to disease traits.[45–49] However, in cases such as prostate cancer (8q24)[50–52] Crohn's disease (16q12, *NOD2*),[53,54] type II diabetes (10q23-q26),[55–57] and MS (5p13),[58,59] some of the strongest associations are observed in regions where linkage has previously been detected and replicated in several studies. Thus, it is relevant to hypothesize that the linkage to chromosome 10q22-q23 region detected consistently in several migraine study samples could represent a region where a relatively highly penetrant and perhaps common variant(s) is/are associated to migraine. Another possibility is that a strong linkage observed in several study samples indicates that there are several susceptibility variants or even genes within the linked locus.

Regardless of the various constraints involved, we detected strong linkage at the 10q22-q23 locus in all three samples assessed in this study. The detection of linkage to the 10q22-q23 locus with different phenotyping methods and different ascertainment protocols provides strong support for the presence of a gene(s) in this region influencing migraine susceptibility. In addition, our study demonstrates the advantages of using of IHS clinical traits directly in migraine genetics and allowed the confirmation of a number of previously reported genomic regions being coinherited with migraine.

Supplemental Data

Two tables are available at http://www.ajhg.org/.

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Web Resources

The URLs for data presented herein are as follows:

matSpD, http://genepi.qimr.edu.au/general/daleN/matSpD/
Migraine Questionnaire used in the SSAGA-1 "Older Cohort," http://genepi.qimr.edu.au/general/daleN/Migraine/SSAGA1MigraineQuestionnaire.pdf
Migraine Questionnaire used in the Twin89 "Younger Cohort," http://genepi.qimr.edu.au/general/daleN/Migraine/Twin89MigraineQuestionnaire.pdf
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
PLINK, http://pngu.mgh.harvard.edu/purcell/plink/
snpSpD, http://genepi.qimr.edu.au/general/daleN/SNPSpD/

References

1. Stovner, L.J., Zwart, J.A., Hagen, K., Terwindt, G.M., and Pascual, J. (2006). Epidemiology of headache in Europe. Eur. J. Neurol. *13*, 333–345.
2. Launer, L.J., Terwindt, G.M., and Ferrari, M.D. (1999). The prevalence and characteristics of migraine in a population-based cohort: The GEM study. Neurology *53*, 537–542.
3. Mulder, E.J., Van Baal, C., Gaist, D., Kallela, M., Kaprio, J., Svensson, D.A., Nyholt, D.R., Martin, N.G., MacGregor, A.J., Cherkas, L.F., et al. (2003). Genetic and environmental influences on migraine: a twin study across six countries. Twin Res. *6*, 422–431.
4. Russell, M.B., and Olesen, J. (1995). Increased familial risk and evidence of genetic factor in migraine. BMJ *311*, 541–544.
5. Andlin-Sobocki, P., Jonsson, B., Wittchen, H.U., and Olesen, J. (2005). Cost of disorders of the brain in Europe. Eur. J. Neurol. *12* (*Suppl 1*), 1–27.
6. Honkasalo, M.L., Kaprio, J., Winter, T., Heikkila, K., Sillanpaa, M., and Koskenvuo, M. (1995). Migraine and concomitant symptoms among 8167 adult twin pairs. Headache *35*, 70–78.
7. Larsson, B., Bille, B., and Pederson, N.L. (1995). Genetic influence in headaches: A Swedish twin study. Headache *35*, 513–519.
8. Nyholt, D.R., Morley, K.I., Ferreira, M.A., Medland, S.E., Boomsma, D.I., Heath, A.C., Merikangas, K.R., Montgomery, G.W., and Martin, N.G. (2005). Genomewide significant linkage to migrainous headache on chromosome 5q21. Am. J. Hum. Genet. *77*, 500–512.
9. Wessman, M., Kallela, M., Kaunisto, M.A., Marttila, P., Sobel, E., Hartiala, J., Oswell, G., Leal, S.M., Papp, J.C., Hamalainen, E., et al. (2002). A susceptibility locus for migraine with aura, on chromosome 4q24. Am. J. Hum. Genet. *70*, 652–662.
10. Lea, R.A., Nyholt, D.R., Curtain, R.P., Ovcaric, M., Sciascia, R., Bellis, C., Macmillan, J., Quinlan, S., Gibson, R.A., McCarthy, L.C., et al. (2005). A genome-wide scan provides evidence for loci influencing a severe heritable form of common migraine. Neurogenetics *6*, 67–72.
11. Anttila, V., Kallela, M., Oswell, G., Kaunisto, M.A., Nyholt, D.R., Hamalainen, E., Havanka, H., Ilmavirta, M., Terwilliger, J., Sobel, E., et al. (2006). Trait components provide tools to dissect the genetic susceptibility of migraine. Am. J. Hum. Genet. *79*, 85–99.
12. Björnsson, A., Gudmundsson, G., Gudfinnsson, E., Hrafnsdottir, M., Benedikz, J., Skuladottir, S., Kristjansson, K., Frigge, M.L., Kong, A., Stefansson, K., and Gulcher, J.R. (2003). Localization of a gene for migraine without aura to chromosome 4q21. Am. J. Hum. Genet. *73*, 986–993.
13. Cader, Z.M., Noble-Topham, S., Dyment, D.A., Cherny, S.S., Brown, J.D., Rice, G.P., and Ebers, G.C. (2003). Significant linkage to migraine with aura on chromosome 11q24. Hum. Mol. Genet. *12*, 2511–2517.
14. Soragna, D., Vettori, A., Carraro, G., Marchioni, E., Vazza, G., Bellini, S., Tupler, R., Savoldi, F., and Mostacciuolo, M.L. (2003). A locus for migraine without aura maps on chromosome 14q21.2-q22.3. Am. J. Hum. Genet. *72*, 161–167.
15. Carlsson, A., Forsgren, L., Nylander, P.O., Hellman, U., Forsman-Semb, K., Holmgren, G., Holmberg, D., and Holmberg, M. (2002). Identification of a susceptibility locus for migraine with and without aura on 6p12.2-p21.1. Neurology *59*, 1804–1807.
16. International Headache Society (1988). Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. Headache Classification Committee of the International Headache Society. Cephalalgia *8*, 1–96.
17. International Headache Society (2004). The International classification of headache disorders: 2nd edition. Cephalalgia *24* (*Suppl 1*), 9–160.
18. Nyholt, D.R., Gillespie, N.G., Heath, A.C., Merikangas, K.R., Duffy, D.L., and Martin, N.G. (2004). Latent class and genetic analysis does not support migraine with aura and migraine without aura as separate entities. Genet. Epidemiol. *26*, 231–244.
19. Blau, J.N. (1995). Migraine with aura and migraine without aura are not different entities. Cephalalgia *15*, 186–190.
20. Ligthart, L., Boomsma, D.I., Martin, N.G., Stubbe, J.H., and Nyholt, D.R. (2006). Migraine with aura and migraine without aura are not distinct entities: Further evidence from a large Dutch population study. Twin Res. Hum. Genet. *9*, 54–63.
21. Kallela, M., Wessman, M., and Färkkilä, M. (2001). Validation of a migraine specific questionnaire for use in family studies. Eur. J. Neurol. *8*, 61–66.
22. Heath, A.C., Bucholz, K.K., Madden, P.A., Dinwiddie, S.H., Slutske, W.S., Bierut, L.J., Statham, D.J., Dunne, M.P., Whitfield, J.B., and Martin, N.G. (1997). Genetic and environmental contributions to alcohol dependence risk in a national twin sample: Consistency of findings in women and men. Psychol. Med. *27*, 1381–1396.
23. Heath, A.C., Howells, W., Kirk, K.M., Madden, P.A., Bucholz, K.K., Nelson, E.C., Slutske, W.S., Statham, D.J., and Martin, N.G. (2001). Predictors of non-response to a questionnaire survey of a volunteer twin panel: Findings from the Australian 1989 twin cohort. Twin Res. *4*, 73–80.
24. Merikangas, K.R., Dartigues, J.F., Whitaker, A., and Angst, J. (1994). Diagnostic criteria for migraine. A validity study. Neurology *44*, S11–S16.
25. Stewart, W.F., Staffa, J., Lipton, R.B., and Ottman, R. (1997). Familial risk of migraine: A population-based study. Ann. Neurol. *41*, 166–172.
26. O'Connell, J.R., and Weeks, D.E. (1998). PedCheck: A program for identification of genotype incompatibilities in linkage analysis. Am. J. Hum. Genet. *63*, 259–266.
27. Abecasis, G.R., Cherny, S.S., Cookson, W.O., and Cardon, L.R. (2002). Merlin–rapid analysis of dense genetic maps using sparse gene flow trees. Nat. Genet. *30*, 97–101.
28. Whittemore, A.S., and Halpern, J. (1994). A class of tests for linkage using affected pedigree members. Biometrics *50*, 118–127.

29. Kong, A., and Cox, N.J. (1997). Allele-sharing models: LOD scores and accurate linkage tests. Am. J. Hum. Genet. *61*, 1179–1188.
30. Weeks, D.E., and Lange, K. (1988). The affected-pedigree-member method of linkage analysis. Am. J. Hum. Genet. *42*, 315–326.
31. Haseman, J.K., and Elston, R.C. (1972). The investigation of linkage between a quantitative trait and a marker locus. Behav. Genet. *2*, 3–19.
32. Elston, R.C., Song, D., and Iyengar, S.K. (2005). Mathematical assumptions versus biological reality: Myths in affected sib pair linkage analysis. Am. J. Hum. Genet. *76*, 152–156.
33. Kruglyak, L., Daly, M.J., Reeve-Daly, M.P., and Lander, E.S. (1996). Parametric and nonparametric linkage analysis: A unified multipoint approach. Am. J. Hum. Genet. *58*, 1347–1363.
34. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., de Bakker, P.I., Daly, M.J., and Sham, P.C. (2007). PLINK: A tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. *81*, 559–575.
35. Lander, E., and Kruglyak, L. (1995). Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. Nat. Genet. *11*, 241–247.
36. Feingold, E., Brown, P.O., and Siegmund, D. (1993). Gaussian models for genetic linkage analysis using complete high-resolution maps of identity by descent. Am. J. Hum. Genet. *53*, 234–251.
37. Rao, D.C., and Province, M.A. (2000). The future of path analysis, segregation analysis, and combined models for genetic dissection of complex traits. Hum. Hered. *50*, 34–42.
38. Abecasis, G.R., Yashar, B.M., Zhao, Y., Ghiasvand, N.M., Zareparsi, S., Branham, K.E., Reddick, A.C., Trager, E.H., Yoshida, S., Bahling, J., et al. (2004). Age-related macular degeneration: A high-resolution genome scan for susceptibility loci in a population enriched for late-stage disease. Am. J. Hum. Genet. *74*, 482–494.
39. Abecasis, G.R., Burt, R.A., Hall, D., Bochum, S., Doheny, K.F., Lundy, S.L., Torrington, M., Roos, J.L., Gogos, J.A., and Karayiorgou, M. (2004). Genomewide scan in families with schizophrenia from the founder population of Afrikaners reveals evidence for linkage and uniparental disomy on chromosome 1. Am. J. Hum. Genet. *74*, 403–417.
40. Suarez, B.K., Duan, J., Sanders, A.R., Hinrichs, A.L., Jin, C.H., Hou, C., Buccola, N.G., Hale, N., Weilbaecher, A.N., Nertney, D.A., et al. (2006). Genomewide linkage scan of 409 European-ancestry and African American families with schizophrenia: Suggestive evidence of linkage at 8p23.3-p21.2 and 11p13.1-q14.1 in the combined sample. Am. J. Hum. Genet. *78*, 315–333.
41. Wiltshire, S., Cardon, L.R., and McCarthy, M.I. (2002). Evaluating the results of genomewide linkage scans of complex traits by locus counting. Am. J. Hum. Genet. *71*, 1175–1182.
42. Ophoff, R.A., Terwindt, G.M., Vergouwe, M.N., van Eijk, R., Oefner, P.J., Hoffman, S.M., Lamerdin, J.E., Mohrenweiser, H.W., Bulman, D.E., Ferrari, M., et al. (1996). Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell *87*, 543–552.
43. De Fusco, M., Marconi, R., Silvestri, L., Atorino, L., Rampoldi, L., Morgante, L., Ballabio, A., Aridon, P., and Casari, G. (2003). Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. Nat. Genet. *33*, 192–196.
44. Dichgans, M., Freilinger, T., Eckstein, G., Babini, E., Lorenz-Depiereux, B., Biskup, S., Ferrari, M.D., Herzog, J., van den Maagdenberg, A.M., Pusch, M., and Strom, T.M. (2005). Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. Lancet *366*, 371–377.
45. Scott, L.J., Mohlke, K.L., Bonnycastle, L.L., Willer, C.J., Li, Y., Duren, W.L., Erdos, M.R., Stringham, H.M., Chines, P.S., Jackson, A.U., et al. (2007). A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science *316*, 1341–1345.
46. Maller, J., George, S., Purcell, S., Fagerness, J., Altshuler, D., Daly, M.J., and Seddon, J.M. (2006). Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration. Nat. Genet. *38*, 1055–1059.
47. Gudmundsson, J., Sulem, P., Steinthorsdottir, V., Bergthorsson, J.T., Thorleifsson, G., Manolescu, A., Rafnar, T., Gudbjartsson, D., Agnarsson, B.A., Baker, A., et al. (2007). Two variants on chromosome 17 confer prostate cancer risk, and the one in TCF2 protects against type 2 diabetes. Nat. Genet. *39*, 977–983.
48. Todd, J.A., Walker, N.M., Cooper, J.D., Smyth, D.J., Downes, K., Plagnol, V., Bailey, R., Nejentsev, S., Field, S.F., Payne, F., et al. (2007). Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat. Genet. *39*, 857–864.
49. Saxena, R., Voight, B.F., Lyssenko, V., Burtt, N.P., de Bakker, P.I., Chen, H., Roix, J.J., Kathiresan, S., Hirschhorn, J.N., et al. (2007). Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science *316*, 1331–1336.
50. Amundadottir, L.T., Sulem, P., Gudmundsson, J., Helgason, A., Baker, A., Agnarsson, B.A., Sigurdsson, A., Benediktsdottir, K.R., Cazier, J.B., Sainz, J., et al. (2006). A common variant associated with prostate cancer in European and African populations. Nat. Genet. *38*, 652–658.
51. Haiman, C.A., Patterson, N., Freedman, M.L., Myers, S.R., Pike, M.C., Waliszewska, A., Neubauer, J., Tandon, A., Schirmer, C., McDonald, G.J., et al. (2007). Multiple regions within 8q24 independently affect risk for prostate cancer. Nat. Genet. *39*, 638–644.
52. Gudmundsson, J., Sulem, P., Manolescu, A., Amundadottir, L.T., Gudbjartsson, D., Helgason, A., Rafnar, T., Bergthorsson, J.T., Agnarsson, B.A., Baker, A., et al. (2007). Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. Nat. Genet. *39*, 631–637.
53. Hugot, J.P., Laurent-Puig, P., Gower-Rousseau, C., Olson, J.M., Lee, J.C., Beaugerie, L., Naom, I., Dupas, J.L., Van Gossum, A., Orholm, M., et al. (1996). Mapping of a susceptibility locus for Crohn's disease on chromosome 16. Nature *379*, 821–823.
54. Rioux, J.D., Xavier, R.J., Taylor, K.D., Silverberg, M.S., Goyette, P., Huett, A., Green, T., Kuballa, P., Barmada, M.M., Datta, L.W., et al. (2007). Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat. Genet. *39*, 596–604.
55. Wiltshire, S., Hattersley, A.T., Hitman, G.A., Walker, M., Levy, J.C., Sampson, M., O'Rahilly, S., Frayling, T.M., Bell, J.I.,

Lathrop, G.M., et al. (2001). A genomewide scan for loci predisposing to type 2 diabetes in a U.K. population (the Diabetes UK Warren 2 Repository): Analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. Am. J. Hum. Genet. *69*, 553–569.

56. Grant, S.F., Thorleifsson, G., Reynisdottir, I., Benediktsson, R., Manolescu, A., Sainz, J., Helgason, A., Stefansson, H., Emilsson, V., Helgadottir, A., et al. (2006). Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat. Genet. *38*, 320–323.
57. Helgason, A., Pálsson, S., Thorleifsson, G., Grant, S.F., Emilsson, V., Gunnarsdottir, S., Adeyemo, A., Chen, Y., Chen, G., Reynisdottir, I., et al. (2007). Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. Nat. Genet. *39*, 218–225.
58. Lundmark, F., Duvefelt, K., Iacobaeus, E., Kockum, I., Wallström, E., Khademi, M., Oturai, A., Ryder, L.P., Saarela, J., Harbo, H.F., et al. (2007). Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. Nat. Genet. *39*, 1108–1113.
59. Hafler, D.A., Compston, A., Sawcer, S., Lander, E.S., Daly, M.J., De Jager, P.L., de Bakker, P.I., Gabriel, S.B., Mirel, D.B., Ivinson, A.J., et al. (2007). Risk alleles for multiple sclerosis identified by a genomewide study. N. Engl. J. Med. *357*, 851–862.