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A Genome-Wide Linkage Scan Provides Evidence for Both New and Previously Reported Loci Influencing Common Migraine

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Latent class analysis was performed on migraine symptom data collected in a Dutch population sample (N = 12,210, 59% female) in order to obtain empirical groupings of individuals suffering from symptoms of migraine headache. Based on these heritable groupings ($h^2 = 0.49$, 95% CI: 0.41–0.57) individuals were classified as affected (*migrainous headache*) or unaffected. Genome-wide linkage analysis was performed using genotype data from 105 families with at least 2 affected siblings. In addition to this primary phenotype, linkage analyses were performed for the individual migraine symptoms. Significance levels, corrected for the analysis of multiple traits, were determined empirically via a novel simulation approach. *Suggestive* linkage for migrainous headache was found on chromosomes 1 (LOD = 1.63; pointwise $P = 0.0031$), 13 (LOD = 1.63; $P = 0.0031$), and 20 (LOD = 1.85; $P = 0.0018$). Interestingly, the chromosome 1 peak was located close to the *ATP1A2* gene, associated with familial hemiplegic migraine type 2 (FHM2). Individual symptom analysis produced a LOD score of 1.97 ($P = 0.0013$) on chromosome 5 (photo/phonophobia), a LOD score of 2.13 ($P = 0.0009$) on chromosome 10 (moderate/severe pain intensity) and a near *significant* LOD score of 3.31 ($P = 0.00005$) on chromosome 13 (pulsating headache). These peaks were all located near regions previously reported in migraine linkage studies. Our results provide important replication and support for the presence of migraine susceptibility genes within these regions, and further support the utility of an LCA-based phenotyping approach and analysis of individual symptoms in migraine genetic research. Additionally, our novel "2-step" analysis and simulation approach provides a powerful means to investigate linkage to individual trait components.

KEY WORDS: migraine; linkage analysis; latent class analysis (LCA); trait components; empirical significance

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INTRODUCTION

Migraine is a severe headache disorder that affects approximately 15% of the population. It has been known for some time that this disorder is under substantial genetic influence. The heritability of migraine is commonly estimated at approximately 50%. To date, genes have only been identified for a rare autosomal dominant subtype of migraine, called familial hemiplegic migraine (FHM). The *ATP1A2* gene on chromosome 1q23 (FHM2) [De Fusco et al., 2003; Vanmolkot et al., 2003], the *SCN1A* gene on chromosome 2q24 [Dichgans et al., 2005], and the *CACNA1A* gene on 19p13 (FHM1) [Joutel et al., 1993; Ophoff et al., 1996] have been implicated in this autosomal dominantly inherited disorder. Evidence is accumulating that the chromosome 1 and 19 loci may also be involved in the common migraines, although more research is required to confirm these findings [Hovatta et al., 1994; May et al., 1995; Ophoff et al., 1997; Nyholt et al., 1998, 2005; Jones et al., 2001; Terwindt et al., 2001; Todt et al., 2005].

Due to the lack of biological markers for migraine, diagnosis relies entirely on symptomatology. The disorder is most commonly diagnosed using the classification criteria proposed by the International Headache Society (IHS) [2004]. The IHS diagnostic criteria are based on clinical consensus, and require patients to have a certain number and combination of symptoms in order to qualify for a migraine diagnosis (Table I). Consequently, a relatively severe form of migraine is required for a positive diagnosis. A study by Lantéri-Minet et al. [2005] showed that, in a large French population sample, the number of subjects qualifying for a "probable migraine" diagnosis (i.e., one feature short of a full migraine diagnosis) was almost as large as the number of subjects fulfilling all criteria. These subjects may not strictly meet the criteria, but are likely to have a genetic liability in common with subjects fulfilling a complete migraine diagnosis. This means that excluding subjects qualifying for a "probable migraine" diagnosis but

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TABLE I. Diagnostic Criteria for Migraine Without Aura, as Published by the International Headache Society [Headache Classification Committee of the International Headache Society, 2004]

Migraine without aura	
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 pain intensity 4. Aggravation by or causing avoidance of routine physical activity (e.g., walking or climbing stairs)
D	During headache at least one of the following: 1. Nausea and/or vomiting 2. Photophobia and phonophobia
E	Not attributed to another disorder

who do not strictly fulfill the IHS migraine diagnosis will lead to a considerable loss of power in genetic studies of migraine.

A method that addresses this issue was proposed by Nyholt et al. [2004]. In this study, latent class analysis (LCA) was applied to the IHS migraine symptom data. Although empirically derived, the resulting groupings of headache sufferers showed similar heritability to strict IHS diagnoses and remain clinically relevant due to being derived from the IHS diagnostic criteria. The LCA method provides a stable and quantitative approach to diagnosing *migrainous headache*, resulting in more individuals being definitively classified, thereby increasing the potential power of genetic studies aimed at identifying genes contributing to the underlying susceptibility of migraine. Good correspondence between LCA-based migraine groupings and genetic risk has been demonstrated [Nyholt et al., 2004; Ligthart et al., 2006]. LCA has now been applied successfully in several migraine studies [Nyholt et al., 2004, 2005; Lea et al., 2005; Ligthart et al., 2006]. In addition, the utility of LCA-based diagnoses and analysis of individual symptoms in genetic studies was recently highlighted in two recent reviews on migraine genetics [van den Maagdenberg et al., 2007; Wessman et al., 2007]. An LCA-based genetic study of migraine in the Dutch population [Ligthart et al., 2006] showed results very similar to those observed in the Australian LCA study by Nyholt et al. [2004], with subgroups of affected individuals differing in the severity rather than the quality of their headaches. In the present study, an LCA-based phenotype (*migrainous headache*) is utilized in a genetic linkage analysis of migraine in a Dutch population sample.

METHODS

Sample

Migraine symptom data were collected in a cohort of Dutch twins and their parents, siblings and partners. The participants were volunteer members of the Netherlands Twin Registry, kept by the department of Biological Psychology at the Vrije Universiteit in Amsterdam. The data were collected in two surveys on health, lifestyle and personality, conducted in 2002 and 2004. Data collection procedures for both surveys have been described in detail elsewhere [Boomsma et al., 2006; Distel et al., 2007]. The 2002 questionnaire data were available for 10,299 individuals (42% males, 58% females) with a mean age of 40.0 (SD = 14.4, range 14–88). The 2004 questionnaire was completed by 8645 individuals (39% males, 61% females) with a mean age of 42.7 (SD = 14.6, range 15–90). Of all participants, 6,631 individuals completed both surveys, resulting in a total number of 12,313 participants across the two surveys. Migraine data were available for 12,210 individuals (5,016 males and 7,194 females) from 4,014 families. Of these individuals, 5,540 (45%) were twins, 1,767 (14%) were singleton siblings of the twins, 3,261 (27%) were parents of twins, and 1,642 (13%) were spouses of twins.

The two surveys both included the same set of headache questions. Participants screening positive for the screening question (do you ever experience headache attacks, for instance migraine?) subsequently answered a set of more detailed headache questions. This information was used to determine the participants' status with regard to eight of the symptoms listed in the IHS diagnostic criteria for migraine (Table II).

All available data were used to determine whether or not each of the symptoms was present in an individual. Between the two questionnaires the tetrachoric test–retest correlation was 0.87 for the screening question, and ranged between 0.79 and 0.91 for the IHS migraine symptoms (assuming individuals screening negative did not have the symptom). Given changes in the presenting symptoms of migraine attacks are common [Ophoff et al., 1994; Kallela et al., 2001], it was assumed that if a participant reports a migraine symptom in one survey but not the other, the presence of that symptom reflects a liability to migraine and is therefore relevant to a study of migraine genetics, even if it was not present a few years earlier or later (i.e., "lifetime" migraine). Therefore, a participant positive for a symptom in either of the two questionnaires was treated as affected with respect to that particular symptom.

Latent Class Analysis

LCA was used to empirically investigate the presence and characteristics of subgroups of headache sufferers in our sample, as previously described in detail [Nyholt et al., 2004; Ligthart et al., 2006]. LCA investigates the relationship between a set of observed variables (in this case migraine symptom data) and an underlying latent (unobserved) construct. The categories of this latent trait are referred to as "clusters" or "classes." Based on the pattern of symptoms reported, the most likely class membership is estimated for each subject [Lazarsfeld and Henry, 1968; McCutcheon, 1987]. In this study, LCA was performed on the individual IHS migraine symptoms, using the software package Latent GOLD 4 (Statistical Innovations Inc., Belmont, MA). Sex was included as a covariate, to allow for differential symptom prevalence in males and females. Model fit was compared using the Bayes Information Criterion (BIC), with a lower BIC indicating a better fit to the data. The empirical groupings resulting from this analysis were used to classify participants as affected or unaffected for "migrainous headache." This

TABLE II. Headache Questions Included in the Surveys and Correspondence to IHS Diagnostic Criteria for Migraine

Question in survey	Code in diagnostic criteria	Description
Do you ever experience headache attacks, for instance migraine? (yes/no)		Screening question
How often do you have these headache attacks?[a]	A	≥5 episodes
Less than once a year		
About once a year		
Several times a year		
About once a month		
Several times a month		
About once a week		
Several times a week		
How long do these headache attacks usually last?	B	4–72 hr
The headache is usually pounding or stabbing (yes/no)	C2	Pulsating quality
How intense is the headache during most attacks? (mild/moderate/severe)	C3	Moderate or severe pain intensity
During a headache attack, do you experience: (yes/no)		
Aggravation of headache by physical activity?	C4	Aggravation by physical activity
Nausea or vomiting?	D1	Nausea and/or vomiting
Aversion of light, sound or smell?[b]	D2	Photo and phonophobia
Partial loss of vision, seeing flashes of light or (zigzag) patterns?		Aura

[a]An attack frequency of at least "several times a year" was assumed to be equivalent to "≥5 episodes."
[b]The official criteria do not include osmophobia and require both photo- and phonophobia, however, from these data it was not possible to determine whether both were present.

classification was used as the primary phenotype in the linkage analyses. The individual migraine symptoms (independent of affection status for migrainous headache) were used as phenotypes in supplementary linkage analyses of implicated regions. Analyzing a broad phenotype at the level of individual symptoms may provide more insight into the relationships between loci and individual symptoms [Nyholt et al., 2005]. More specifically, by analyzing symptoms independent of the endpoint diagnosis, within-family phenotypic homogeneity is typically increased. For example, although not all subjects may be classified as affected for the end diagnosis they may nonetheless all suffer a particular symptom.

For comparison purposes only, results for migraine diagnosed according to strict IHS criteria (see Table I) are also reported. The heritability and 95% confidence intervals for migrainous headache based on LCA and IHS criteria were estimated with Mx [Neale et al., 2003], using all available twin data.

Genotype Data

DNA was extracted from either whole blood or buccal swabs using standard protocols [Miller et al., 1988; Meulenbelt et al., 1995]. Samples were genotyped by the Mammalian Genotyping Service in Marshfield and the Molecular Epidemiology Section, Leiden University Medical Centre. The genotype data from these genome-wide and some candidate region screens were combined to a single data set where alleles of the same markers between sets were aligned. Pedigree relationships were examined using GRR (Graphic Representation of Relationships) and errors of Mendelian inheritance were detected with Pedstats [Abecasis et al., 2001, 2002]. Markers and samples were removed if their total error rate was more than 1%; in all other cases the specific erroneous genotypes were coded as unknown. Merlin was used to detect unlikely recombinants and erroneous genotypes were removed with Pedwipe [Abecasis et al., 2002].

The siblings from the families informative for linkage had an average of 345 markers typed. The average marker spacing per individual had a median of 10 cM, and the average heterozygosity of the autosomal markers was 75%. Sex-averaged, female- and male-specific map positions were interpolated via locally weighted linear regression from NCBI build 35.1 physical map positions and the Rutgers genetic map [Duffy, 2006] (http://www2.qimr.edu.au/davidd/master_map.dat).

Linkage Analysis

Multipoint "non-parametric" linkage analysis was performed using Merlin [Abecasis et al., 2002]. The NPL_{pairs} statistic [Weeks and Lange, 1988] was used to test for increased allele sharing among affected individuals. The genotyped sample consisted of 3,944 individuals from 841 nuclear families. For 2,536 of these migraine data were available. Informative for linkage were all genotyped families in which at least two siblings were affected. Under the LCA-based definition of *migrainous headache*, 105 nuclear families were informative, encompassing 234 affected, and 73 unaffected siblings. Allowing for non-independence among sib pairs derived from the same sibship (i.e., sibship of size S as being equivalent to S-1 independent sib pairs [Suarez and Hodge, 1979]), these 105 families contained 202 independent sib pairs, 129 of which were affected concordant (of the remaining sibling pairs in the larger families, 25 were unaffected concordant and 48 were discordant). These numbers vary for the individual symptoms, which have different prevalences (113 informative families for ≥5 attacks, 60 for 4–72 hr duration, 49 for pulsation, 108 for moderate/severe, 74 for aggravation, 40 for nausea/vomiting, 69 for photo-/phonophobia, and 16 for visual aura).

LOD scores were calculated according to the Kong and Cox exponential model [Kong and Cox, 1997]. Regions in which LOD scores exceeded the threshold for suggestive linkage were further explored, using the individual migraine symptoms (i.e., the presence of a symptom, regardless of LCA diagnosis) as phenotypes. Finally, to ensure that no important findings were missed due to our focus on suggestive regions only, a genome-wide exploratory analysis was carried out for all the phenotypes.

Empirical estimates of genome-wide significance were obtained via gene-drop simulations performed using Merlin. Based on the observed phenotype and genotype data, 1,000 "null" genome scans were generated under the assumption of no linkage. The simulated genome scans were analyzed in the same way as the original data. From each analysis the highest LOD score per chromosome was collected. The empirical

significance of a LOD score was determined by counting the proportion of genome scans containing LOD scores that exceeded that value. Following the recommendations of Lander and Kruglyak [1995], the threshold for *suggestive* linkage was defined as the LOD score that occurred by chance only once per genome scan, in other words, the 1,000th highest LOD score in a total of 1,000 simulated genome scans (LOD $\geq$ 1.54 in the current data). *Significant* linkage was defined as a LOD score that occurs with probability 0.05 in a genome scan, or once per 20 genome scans. This is equivalent to the 50th highest LOD score occurring in 1,000 simulated scans (LOD $\geq$ 2.82 in the current data).

The empirical significance values for the follow-up analyses of the suggestive regions, which included the individual symptoms as phenotypes, had to be corrected for multiple testing. This can be done by analyzing the simulated genome scans for all nine phenotypes, and collecting for each position the highest LOD score across these phenotypes. Out of these "maximized" LOD scores, the highest LOD per chromosome was recorded. This was done for each of the 1,000 replicates. As in the procedure described above, the suggestive (LOD $\geq$ 2.4) and significant (LOD $\geq$ 3.85) linkage threshold was taken as the 1,000th and 50th highest "maximized" LOD scores, respectively. This procedure was used to determine empirical significance levels for the exploratory genome-wide linkage analyses of LCA migrainous headache and the individual symptoms.

However, given our primary strategy was to only follow up the regions that showed suggestive linkage with migrainous headache, correcting for analyzing nine phenotypes genome-wide would lead to a conservative significant linkage threshold. Therefore, we developed a novel "2-step" simulation approach which examined the "maximized" LOD score, given analysis of nine phenotypes, only in regions showing suggestive linkage with migrainous headache. More specifically, for each simulated genome-wide linkage scan the "maximized" LOD score across all phenotypes was calculated only for the simulated chromosomes in which the simulated LOD score for migrainous headache exceeded our initial suggestive linkage threshold (LOD $\geq$ 1.54). Analysis of the resulting "maximized" LOD scores enables determination of a *significant* linkage threshold corrected for our restricted testing of multiple phenotypes. This significance level (LOD $\geq$ 3.57) will be referred to as "2-step" significant linkage.

RESULTS

LCA was performed using migraine symptom data from 12,210 individuals (41% male, 59% female). Sex was included in the models as a covariate. A five-class LCA model provided the best fit to the data, with the minimum BIC value of 52067.28 (four- and six-class models produced larger BIC values of 52173.47 and 52117.74, respectively). Figure 1 shows the resulting profile plot, depicting the endorsement probability for each symptom given class membership. The subjects screening negative (67%, N = 8,138, 48% male, 52% female; data not shown) did not answer any further questions about headache and were assumed to be unaffected for all migraine-related symptoms. Therefore the endorsement probabilities for this group were zero. The two least severe symptomatic categories (5%, N = 647, 48% male, 52% female) can be described as "mild non-migrainous headache." These two categories are referred to as class 1a and 1b because they are relatively similar in both quality and severity. Typically, individuals in these classes were unaffected (i.e., had low endorsement probabilities) for the majority of IHS migraine symptoms. The individuals in class 2 (12%, N = 1,481, 36% male, 64% female) had a moderately severe type of migrainous headache, typically characterized by the presence of four or more IHS migraine symptoms, but often lacking nausea and/or vomiting, photo- and phonophobia, and/or aura symptoms. The individuals in the most severely affected subgroup (class 3; 16%, N = 1,944, 13% males, 87% females) can be described as having "severe migrainous headaches," typically including the majority of IHS migraine symptoms.

In the genetic analyses, subjects in classes 2 and 3, who, on average, had endorsement probabilities higher than 0.5 for the majority of symptoms (28.1% of the sample), were treated as "affected" for migrainous headache. Subjects in classes 0 and 1 were considered "unaffected." This classification, which is the

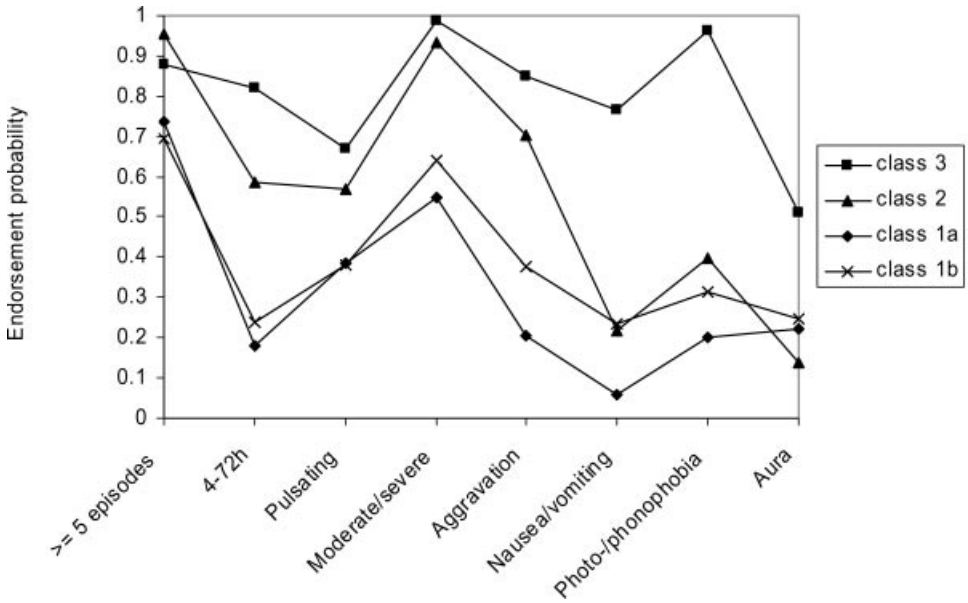

Fig. 1. Profile plot for the 5-class LCA model. The endorsement probabilities (*y*-axis) indicate the proportion of individuals within a class reporting each symptom. In class 0 (not shown), all endorsement probabilities were zero.

primary phenotype in our analyses, will be referred to as "LCA migrainous headache" throughout the paper. To enable a comparison with a more strict definition of migrainous headache, we also report linkage results for an LCA-based classification in which only class 3 individuals are treated as affected ("LCA-severe," 16% of the sample) and for a classification based on strict IHS criteria ("IHS migraine," 12.2% of the sample). Table III shows the number of individuals with IHS migraine by class membership.

Maximum likelihood estimates of heritability were obtained in Mx, using all available twin data (N = 2,036 pairs, 1,127 MZ, 532 DZ same sex, 377 DZ opposite sex). No significant sex differences in genetic architecture were observed. The heritability of LCA migrainous headache (49%; 95% CI: 41–57) was slightly higher and more precise than the heritability of IHS migraine (46%; 95% CI: 36–56) and LCA-severe (43%; 95% CI: 33–52). This indicates that the LCA migrainous headache phenotype provides at least a similar amount of genetic information compared to the stricter IHS and LCA-severe diagnoses, while also increasing the number of individuals classified as affected for migrainous headache.

LCA migrainous headache was subsequently utilized as the primary phenotype in genome-wide linkage analysis. Figure 2a shows the LOD scores from Merlin NPL_{pairs} analysis of LCA migrainous headache. For comparison, linkage results using the LCA-severe and IHS migraine classifications are shown in Figure 2b.

The linkage analysis of LCA migrainous headache revealed three LOD scores that exceeded the threshold for suggestive linkage (LOD $\geq$ 1.54). The highest peak was found on chromosome 20, at 41 cM (LOD = 1.85). On chromosome 1, a LOD score of 1.63 was found at 171 cM, and a LOD score of 1.63 was found at 91 cM on chromosome 13. These suggestive linkage regions were subsequently investigated in a multiple phenotype analysis of the individual migraine symptoms (Fig. 3). Figure 3 also presents results from exploratory linkage analysis of individual symptoms for chromosomes 5 and 10—previously linked to LCA-derived migrainous headache in an Australian sample [Nyholt et al., 2005]. On chromosomes 1 and 20, analyzing the individual symptoms did not result in higher LOD scores compared to analyzing migrainous headache only. In contrast, analysis of chromosome 13 produced a considerably higher LOD score for "pulsating headache." The peak (LOD = 1.63) that reached the suggestive linkage threshold for migrainous headache increased to a LOD score of 3.31 in the analysis of pulsating headache—just falling short of our "2-step" significant linkage threshold of 3.57. A neighboring peak for the same symptom, located ~20 cM away, reached a LOD score of 3.34 in the individual symptom analysis.

Finally, to exclude the possibility that linkage to individual migraine symptoms was missed by only examining regions reaching suggestive linkage to migrainous headache, a genome-wide analysis was performed for migrainous headache and all individual symptoms. Figure 4 shows the "maximized" LOD scores across all phenotypes for each position in the genome. No new peaks were identified that exceeded the empirically determined threshold for suggestive (LOD $\geq$ 2.4) or significant linkage (LOD $\geq$ 3.85), after correcting for exploratory analysis of multiple phenotypes genome-wide.

TABLE III. Number of Affected and Unaffected Individuals Based on LCA Classification and IHS Criteria

	IHS diagnosis	
LCA diagnosis	Unaffected	Affected
Class 0 (unaffected)	8138 (66.6%)	0 (0.0%)
Class 1 (unaffected)	644 (5.3%)	3 (0.0%)
Class 2 (affected)	1191 (9.8%)	290 (2.4%)
Class 3 (affected)	743 (6.1%)	1201 (9.8%)

DISCUSSION

Linkage analysis of our primary phenotype, LCA migrainous headache, resulted in three peaks that exceeded our threshold for suggestive linkage, whereas only one such peak was expected to occur by chance. To enable comparison with previously reported linkage results for migraine, an overview of previous studies is given in Table IV. One suggestive peak, with a LOD score of 1.63 (pointwise $P = 0.0031$) at marker D1S1653, was found on chromosome 1, only 5 cM from the *ATP1A2* gene, which has been demonstrated to play an important role in FHM2. This finding is especially interesting since in an Australian study, Nyholt et al. [2005] found a LOD score of 1.53 in the same region. This is further evidence in support of the hypothesis that the FHM2 gene *ATP1A2* or a flanking gene may be involved in common migraine. The fact that numerous families contributed towards this linkage peak indicates it is unlikely that the signal was caused by potential FHM families, considering the low prevalence of this disorder. Another suggestive peak was located on chromosome 13. This peak increased substantially in the individual symptom analysis, with pulsating headache producing the highest LOD score of 3.31 (pointwise $P = 0.00005$). Although the chromosome 13 peak is broad, covering a wide area of the chromosome, it is interesting to note that the highest LOD score found in the individual symptoms analysis is located only 6 cM away from the locus where Nyholt et al. [2005] found suggestive linkage for LCA migraine and the individual photophobia symptom. That said, the broadness of the chromosome 13 peak may indicate the presence of multiple migraine susceptibility loci; however, further research is required to either confirm or exclude this possibility.

Although our final analysis, which included all phenotypes genome-wide, did not reveal any undiscovered peaks exceeding the threshold for suggestive linkage, a few results are worth mentioning. A potentially interesting finding is the linkage for moderate/severe pain intensity on chromosome 10 (highest LOD = 2.13, nominal pointwise $P = 0.0009$), approximately 30 cM away from the linkage peak (marker D10S2327), but overlapping the 95% CI, in the region reported in Australian (highly suggestive linkage for LCA migraine) and Finnish (nearly suggestive linkage for phonophobia) genome scans [Nyholt et al., 2005; Anttila et al., 2006]. In addition, on chromosome 5, a LOD score of 1.97 (pointwise $P = 0.001$) was found for photo-/phonophobia, at marker D5S2501. This replicates the significant linkage found by Nyholt et al. [2005] for LCA migraine, at the same marker. It should be emphasized that, in the absence of identified predisposing genes for common migraine, linkage findings are our main source of information, and replication of these findings is crucial to be able to distinguish between true loci and false positive findings.

The phenotype was based on a questionnaire that included information on 8 of the symptoms listed in the IHS diagnostic criteria for migraine. Since the questionnaire did not include a question about unilateral location of headaches (one of the four C-criteria in the IHS guidelines, see Table I), a complete IHS-based diagnosis was not possible. To avoid false positive migraine diagnoses due to missing symptom data, a slightly more strict definition was used, in which patients were required to have at least two of the three available C-criteria. This may have led to a slightly conservative estimate of IHS migraine prevalence. The possibility cannot be excluded that the absence of information on unilateral location may have also affected the LCA results. However, in a study of similar design

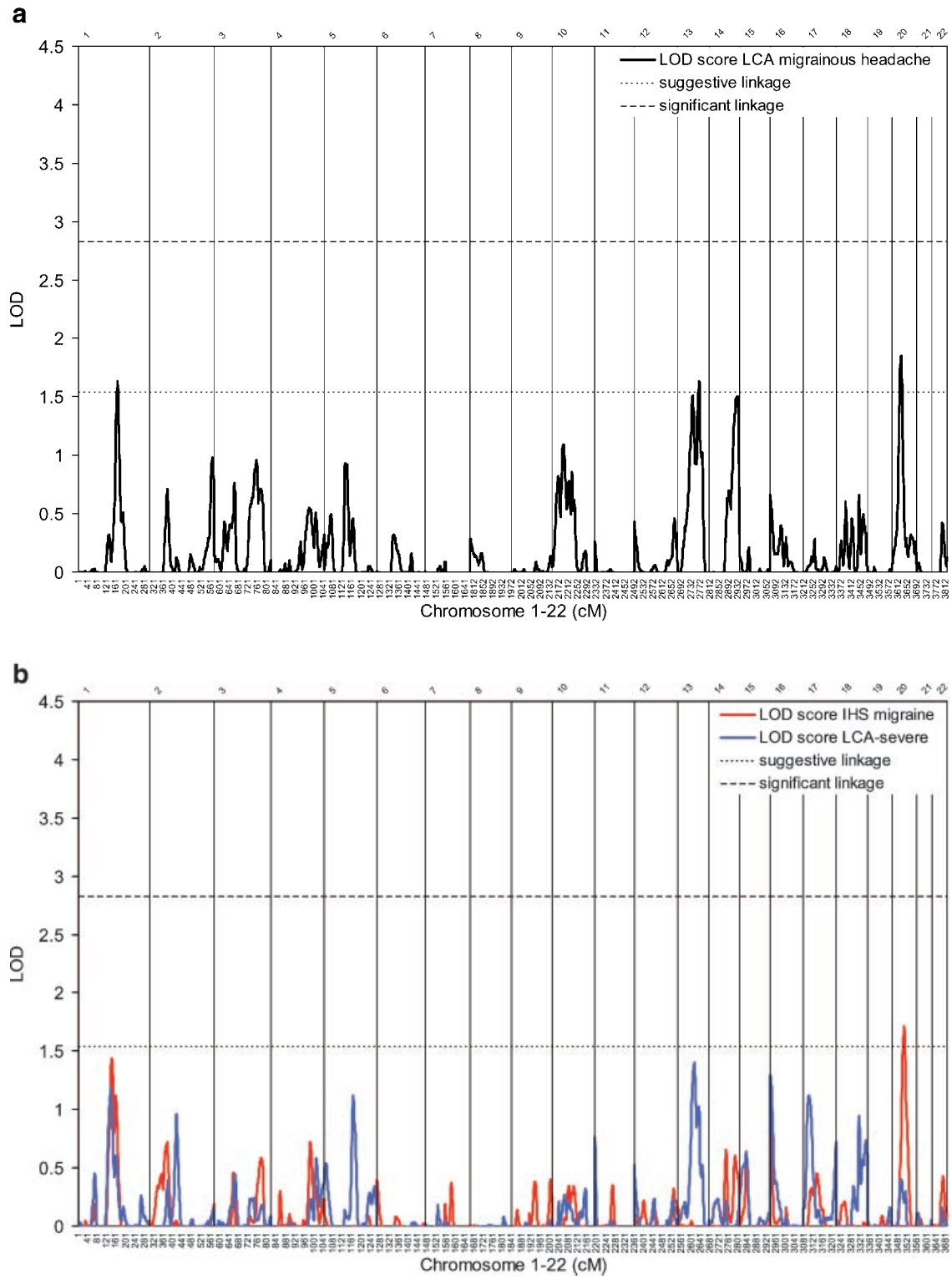

Fig. 2. Chromosome 1-22, LOD scores and empirical significance levels for linkage analysis of (**a**) the primary phenotype, LCA migrainous headache and (**b**) IHS migraine and LCA-severe. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

[Nyholt et al., 2004], unilateral headache was found to be one of the features least distinctive of migraine (as opposed to non-migrainous headache). Therefore it is not expected that the presence of information on unilaterality would have significantly changed the resulting classification.

The primary linkage analyses were performed using a relatively broad definition of migrainous headache. To examine the effects of including individuals with milder forms of migrainous headache, we performed additional linkage analyses on two more strictly defined phenotypes, LCA-severe

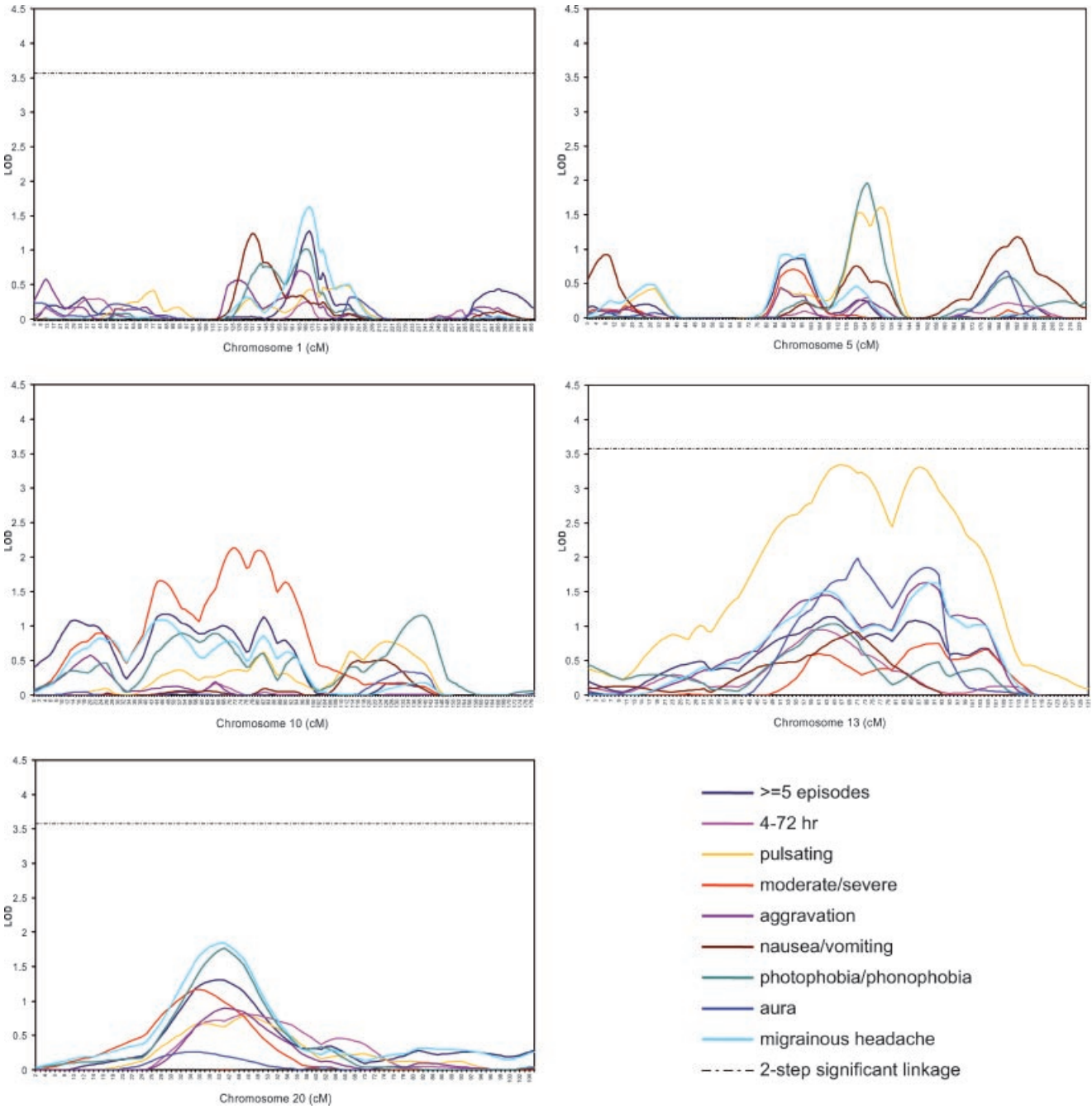

Fig. 3. LOD scores for LCA migrainous headache and individual migraine symptoms; chromosomes 1, 5, 10, 13, and 20. The 2-step significance level is indicated for the regions showing suggestive linkage in the primary analysis.

and IHS migraine (Fig. 2b). Under the stricter classifications, the number of informative families was dramatically reduced (to 48 for LCA-severe and to 32 for IHS migraine). This is due to the fact that even if only one sibling is unaffected under the new classification, both members of the pair are no longer informative for linkage. Figure 2 shows that although the location of the main peaks do not change substantially, the linkage peaks for the stricter definitions are generally lower. Under the IHS definition, the chromosome 13 peak has disappeared entirely, whereas under the LCA-severe definition, the chromosome 13 peak is present but the peak on chromosome 20 is much smaller. Such reductions in LOD scores are expected with a reduction in sample size (i.e., reduced power).

Additional linkage analysis of chromosome 13, for (a) families informative for IHS migraine and (b) families informative for LCA migrainous headache but not IHS migraine, showed that the latter families are indeed responsible for the chromosome 13 peak (results not shown). In other words, the observed linkage to chromosome 13 is predominantly due to increased allele sharing (IBD) amongst individuals suffering moderate migrainous headaches that do not quite satisfy IHS diagnostic

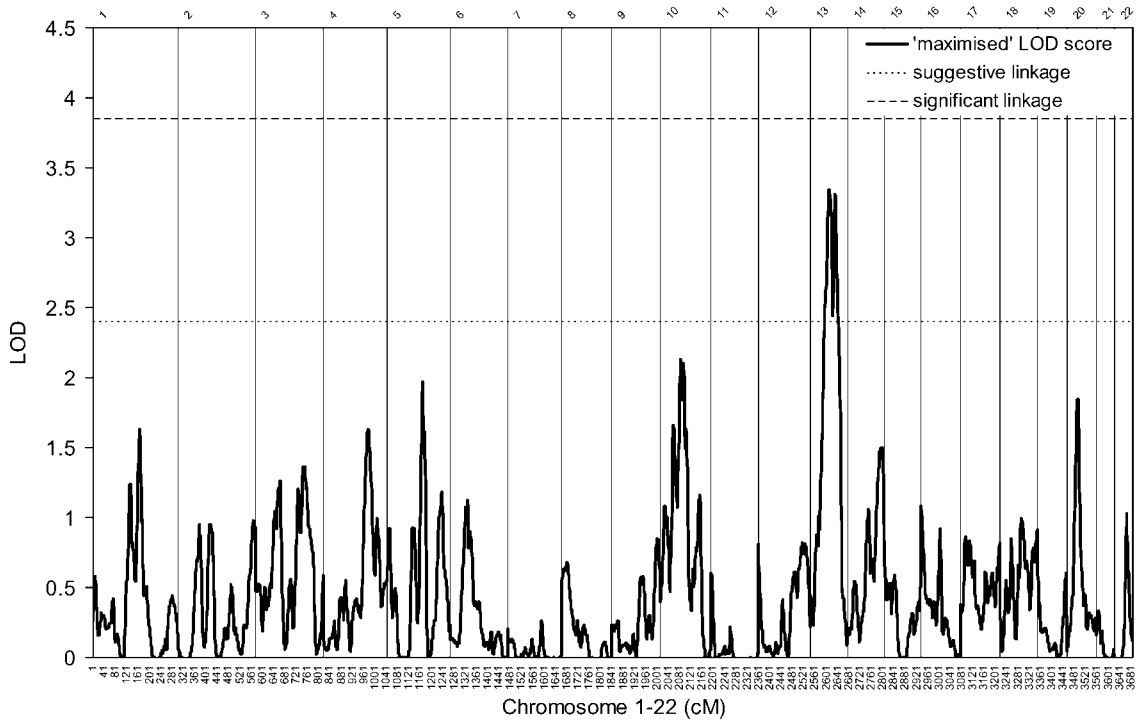

Fig. 4. Chromosome 1-22, genome-wide "maximized" LOD scores across all nine phenotypes (LCA migrainous headache and individual migraine symptoms).

criteria. Closer inspection showed that the general frequency of the symptoms was lower in these families, but the overall pattern of symptoms was very similar to that in the families informative for IHS migraine.

The agreement (Cohen's kappa) between LCA migrainous headache and IHS migraine was 0.53. This kappa is quite good in light of the ambiguity associated with the clinical diagnosis of migraine. For example, kappas range from 0.55 to 0.81 among neurologists assigning headache diagnoses based on videotaped patient interviews [Granella et al., 1994]. Disagreement comes mainly from individuals who are unaffected under the IHS classification but affected under the LCA classification (Table III). This is not limited to the less severely affected class 2 individuals; a substantial number of class 3 individuals were also classified as unaffected under the IHS definition.

The comparison between LCA migrainous headache (classes 2 and 3), and LCA-severe (class 3 only) further supports the idea that gene-finding studies may benefit from the inclusion of individuals with mild migrainous headaches. The linkage results for the two phenotypes are globally similar, but peaks present in the results for LCA-severe tend to increase when class 2 individuals are also included. Inspection of the sibling pairs excluded from the analysis of LCA-severe showed that in most cases one sibling was in class 2 and the other in class 3. Although in such cases the class 2 sibling will have a milder form of headache, the two siblings are likely to have a genetic risk in common, which means that including class 2 siblings adds valuable genetic information.

Our results indicate that the loss of power resulting from exclusion of subjects with milder forms of migrainous headache is unnecessary. Eventually, in genetic studies we are interested in genes underlying a disorder that covers a broad spectrum of severity, and not only in cases exceeding a particular clinical threshold. Although the LCA approach might not necessarily be the most appropriate strategy in clinical practice, our results support the idea that in genetic studies it may be more effective than conventional phenotyping based on strict IHS diagnostic criteria.

Finally, we emphasize the advantage of our novel "2-step" analysis and simulation approach, which only examines individual symptoms in regions surpassing suggestive linkage, compared to an approach analyzing all phenotypes genome-wide. That is, the "2-step" approach ensures those regions initially reaching suggestive linkage remain so, because here we are simply investigating the chance of obtaining *significant* linkage, after examining the individual symptoms in the *suggestive* regions; whereas analysis of all phenotypes genome-wide, carries with it a higher multiple test burden. Indeed, sole use of the latter exploratory approach would result in only the chromosome 13 locus reaching suggestive linkage. Given our goal of analyzing individual symptoms is to increase the evidence for linkage at particular loci, the "2-step" approach makes conceptual sense since it is unlikely a non-suggestive linkage peak obtained for an end diagnosis would reach *significant* linkage for an individual symptom.

In summary, two of our suggestive peaks were located close to loci previously reported in migraine research. To our knowledge, the suggestive peak on chromosome 20 has not been reported before. This study also replicated the significant peak on chromosome 5q21 [Nyholt et al., 2005] and the highly suggestive peak on 10q22 [Nyholt et al., 2005; Anttila et al., 2006]. These results provide important replication and support for the presence of migraine susceptibility genes within these regions, and will be useful in guiding future research efforts in the area of gene-finding. This aspect will become even more important with the recent development towards genome-wide association studies. Linkage results, especially when replicated, can serve as important guidelines for the interpretation of the enormous amounts of data generated by such large-scale genotypic analyses.

Furthermore, the remarkable similarities of LCA classification results and subsequent linkage findings in the genetically similar Dutch and Australian populations [Sullivan et al.,

TABLE IV. Main Results of Genome-Wide Linkage Studies for Migraine, and Results of the Present Study at Previously Reported Loci

Previous study					Current study				
								N subjects (genotyped)	
Refs.	Phenotype	Reference marker	LOD score	Nearest marker	cM from reference marker	LOD score	Phenotype	N affected	N families
Nyholt et al. [2005]	Phonophobia	D1S1679	1.79[a]	D1S1679	0	1.01	LCA migrainous headache	556	756
Lea et al. [2005]	LCA-severe	D3S1311	2.28[a]	D3S1311	0	0.56	Aura	380	92
Bjornsson et al. [2003]	"Loose" MO (females only)	D4S2409	4.08[a]	D4S1534	0.58	0.31	Aggravation	203	77
Anttila et al. [2006]	Age at onset	D4S1647	4.52[b]	D4S1647	0	0.40	Aggravation	225	50
Wessman et al. [2002]	MA	D4S1647	4.2[b]	D4S1647	0	0.40	Aggravation	246	50
Nyholt et al. [2005]	LCA migraine	D5S2501	3.7[a]	D5S2501	0	1.97	Photo/phonophobia	556	756
Carlsson et al. [2002]	MO and MA	D6S452	5.41[b]	D6S2410	3.30	0.61	Moderate/severe	30	1
Nyholt et al. [2005]	LCA migraine	D8S270	1.77[a]	D8S270	0	−0.01	Aggravation	556	756
Nyholt et al. [2005]	LCA migraine	D10S2327	2.32[a]	D10S2327	0	0.47	Moderate/severe	556	756
Anttila et al. [2006]	Phonophobia	D10S2327	2.27[b]	D10S2327	0	0.47	Moderate/severe	225	50
Cader et al. [2003]	MA	D11S4464	4.24[b]	D11S4464	0	0.00	Nausea/vomiting	248	43
Nyholt et al. [2005]	LCA migraine	D13S1807	1.63[a]	D13S1807	0	2.79	Pulsating	556	756
Soragna et al. [2003]	MO	D14S978	3.70[b]	GATA90G11	3.34	0.14	Pulsating	21	1
Anttila et al. [2006]	Pulsation	D17S945	4.65[b]	D17S1852	2.12	0.80	Photo/phonophobia	225	50
Lea et al. [2005]	LCA-severe	D18S53	2.32[a]	D18S53	0	0.82	≥5 attacks	380	92
Anttila et al. [2006]	IHS full criteria	D18S877	3.29[b]	D18S877	0	0.13	≥5 attacks	225	50

MO, migraine without aura; MA, migraine with aura.
[a]Non-parametric multipoint.
[b]Parametric two-point.

2006], indicate that our LCA-based approach provides a stable and robust migraine phenotype and should further encourage the use of this strategy in future migraine genetic research.

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