Hum Genet (2009) 125:591–604
DOI 10.1007/s00439-009-0652-7

ORIGINAL INVESTIGATION

Linkage and heritability analysis of migraine symptom groupings: a comparison of three different clustering methods on twin data

Carla C. M. Chen · Kerrie L. Mengersen · Jonathan M. Keith · Nicholas G. Martin · Dale R. Nyholt

Received: 11 January 2009 / Accepted: 6 March 2009 / Published online: 19 March 2009

Abstract Migraine is a painful disorder for which the etiology remains obscure. Diagnosis is largely based on International Headache Society criteria. However, no feature occurs in all patients who meet these criteria, and no single symptom is required for diagnosis. Consequently, this definition may not accurately reflect the phenotypic heterogeneity or genetic basis of the disorder. Such phenotypic uncertainty is typical for complex genetic disorders and has encouraged interest in multivariate statistical methods for classifying disease phenotypes. We applied three popular statistical phenotyping methods—latent class analysis, grade of membership and grade of membership "fuzzy" clustering (Fanny)—to migraine symptom data, and compared heritability and genome-wide linkage results obtained using each approach. Our results demonstrate that different methodologies produce different clustering structures and non-negligible differences in subsequent analyses. We therefore urge caution in the use of any single approach and suggest that multiple phenotyping methods be used.

C. C. M. Chen (✉) · K. L. Mengersen · J. M. Keith
School of Mathematical Sciences,
Queensland University of Technology,
Brisbane, QLD 4001, Australia
e-mail: carla.chen@qut.edu.au

K. L. Mengersen
e-mail: k.mengersen@qut.edu.au

J. M. Keith
e-mail: j.keith@qut.edu.au

N. G. Martin
Genetic Epidemiology Laboratory,
Queensland Institute of Medical Research,
PO Royal Brisbane Hospital, Brisbane,
QLD 4029, Australia
e-mail: Nick.Martin@qimr.edu.au

D. R. Nyholt
Neurogenetics Laboratory and Genetic Epidemiology Laboratory, Queensland Institute of Medical Research,
PO Royal Brisbane Hospital, Brisbane, QLD 4029, Australia
e-mail: daleN@qimr.edu.au

Introduction

The essential first step for linkage analysis or association studies is to accurately identify the phenotype. For complex diseases such as migraine, identification of the phenotype is challenging due to the lack of objective markers and uncertainty about the etiology of the disease. The diagnosis of this type of disorder is often based on satisfaction of clinically accepted criteria. Although they may not be useful for diagnosis and treatment, these clinical-based phenotypes may not be optimal for genetic research, in particular finding genetic loci contributing to disease inheritance (eg., Hallmayer et al. 2003) and this has led to a call for the development and use of new phenotyping strategies in genetic research (e.g., Wessman et al. 2007).

Migraine is a common, painful and debilitating disorder. Numerous researchers have shown that there is a significant genetic component to risk of this disorder (Ziegler et al. 1998; Mulder et al. 2003; Svensson et al. 2003, 2004; Nyholt et al. 2004, 2005), with estimates of heritability ranging between 34 and 57% in twin-cohort studies across six countries (Mulder et al. 2003). The diagnosis of migraine is found to be difficult due to lack of biological markers and overlap with other types of neurological disorders, such as tension type headache and brain tumour.

To date, the diagnosis of migraine relies on classifying self-reported headache characteristics using International Headache Society (IHS) criteria (Headache Classification Committee of the International Headache Society 1988; Olesen and Steiner 2004; Silberstein et al. 2005). These criteria were developed for standardising headache definition (e.g., Ligthart et al. 2006). The two major subtypes of migraine are migraine without aura (MO) and migraine with aura (MA); the definitions of both types are listed in Tables 1 and 2, respectively.

These criteria have improved migraine diagnosis and subsequently, epidemiological research. However, none of the features occur in all patients who meet a strict definition of IHS migraine, and no single symptom is required for diagnosis. In other words, migraine is a complex of symptoms with variable symptom profiles and individuals presenting with dissimilar symptoms can equally satisfy the same diagnosis. Furthermore, although individuals may not quite satisfy IHS criteria they would nonetheless be treated as such in a clinical setting; indeed there is an IHS classification of "probable migraine" (previously termed "migrainous disorder not fulfilling the above criteria"). The majority of genetic studies for migraine to date have concentrated on either MO or MA and found various chromosome regions associated with each (Table 3). Under these phenotype definitions, no common gene was replicated across studies. However, when migraine phenotypes were identified using a statistical (rather than medical) phenotyping classification via latent class analysis, Ligthart et al. (2008) successfully replicated two susceptibility loci: chromosome 5q21 and 10q22–q23 (Nyholt et al. 2005; Anttila et al. 2006, 2008; Ligthart et al. 2008).

A wide variety of statistical methods have been employed to identify clusters and classes based on symptomatic data. Classical methods such as principal component analysis (PCA) and discriminant analysis (DA) have previously been used in genetic linkage analysis. However, these approaches assume individuals belong to only one of potentially many clusters, which may neglect the phenotypic heterogeneity present in complex human diseases (Kaabi and Elston 2003; Manton et al. 2004). In contrast, "fuzzy" clustering such as latent class analysis (LCA) and grade of membership (GoM) resolve the

Table 1 The 1988 International Headache Society diagnostic criteria for migraine without aura (MO)

Item	Description
A	At least five attacks fulfilling B–D
B	Headache attacks lasting 4–72 h
C	Headache has at least two of the following characteristics:
	Unilateral Locations
	Pulsating quality
	Moderate or severe intensity(inhibits or prohibits daily activities)
	Aggravation by walking stairs or similar routine physical activity
D	During headaches at lease one of the following:
	Nausea and (or) vomiting
	Photophobia and phonophobia

Table 2 The 1988 International Headache Society diagnostic criteria for migraine with aura (MA)

Item	Description
A	Headache fulfilling criteria B–D list in Table 1
B	At least five attacks fulfilling B–D
C	Aura consisting of at least one of the following but no motor sickness
	Fully reversible visual symptoms including positive features (i.e. flicking of lights) and (or) negative features (i.e. loss of vision)
	Fully reversible sensory symptoms including positive (i.e. pins and needles) and (or) negative features (i.e. numbness)
	Fully reversible dysphasic speech disturbance
D	At least two of the following:
	Homonymous visual symptoms and (or) unilateral sensory symptoms
	At least one of the aura symptom develops gradually over ≥5 min
	Each symptom lasts ≥5 min and ≤60 min

Table 3 Table showing the significant linkage signals which are identified in the literature for IHS criteria defined migraine with aura (MA) and migraine without aura (MO)

Phenotype	Cohort	Chromosome	References
MO	Icelandic	4q21	Björnsson et al. (2003)
MO	Italian	14q21.2–q22.3	Soragna et al.(2003)
MA	Canadian	11q24	Cader et al. (2003)
MA	Finnish	4q24	Wessman et al. (2002)
MA and MO	Sweden	6p12.2–p21.1	Carlsson et al. (2002)
MA*	Finnish and Australian	10q22–q23	Anttila et al. (2008)

* Including three types of migraine with aura

–Pure MA, individuals fulfilling IHS criteria for migraine with aura

–Unclassified MA, a group of individuals that cannot be grouped into the IHS defined categories, but clearly suffer from aural features

–Mixed migraine, a group of individuals that commonly have both MA and MO type of attacks

heterogeneity by assigning individuals to multiple clusters and quantified measures of the probability of belonging to each group.

Latent class analysis (McCutcheon 1987) has been widely used in subtyping complex diseases such as migraine (Nyholt et al. 2004, 2005), attention-deficit/hyperactivity disorder (ADHD) (Volk et al. 2005) and schizophrenia (Jablensky 2006) in the field of genetics. Another type of fuzzy clustering, Grade of Membership (GoM), has also been frequently used to obtain empirical phenotypes. This clustering method was first used for medical classification in 1978 (Woodbury et al. 1978) and is now commonly employed for disease subtyping. It has been employed in genetic research for diseases with complex etiology (Cassidy et al. 2001; Corder and Woodbury 1993; Fillenbaum 1998; Manton et al. 1994).

Most recently, Kaabi and Elston (2003) proposed a different type of clustering method which is also called Grade of Membership (GoM). Unlike the model-based GoM proposed by Woodbury et al. (1978), the method suggested by Kaabi and Elston (2003) is based on partitioning the data into a pre-determined number of clusters. To avoid confusion in nomenclature, the grade of membership proposed by Kaabi and Elston (2003) will be referred to as Fanny (Kaufman and Rousseeuw 1990) in this paper. This method has been used to identify loci causing anxiety disorder (Kaabi et al. 2006).

Although some literature has compared the mathematical and statistical differences between LCA and GoM (Manton et al. 1994; Potthoff et al. 2000; Erosheva 2002b, 2005), the effects of these three common phenotyping methods, LCA, GoM and Fanny in genetic analyses such as heritability and genome-wide linkage have not been investigated. Therefore, the aim of this study is to (1) compare these three methods as they apply to common migraine symptomatic twin data, (2) benchmark their performance in genetic research and (3) investigate whether different clustering methods result in different loci being implicated in linkage analysis.

Materials and methods

The symptomatic data were first analyzed by three different phenotyping methods: latent class analysis (LCA), grade of membership (GoM) and fuzzy clustering (Fanny) to obtain a continuous (quantitative) phenotype trait (score) for individuals. The value of phenotypic measures derived from these three models was constrained to be between 0 and 1, which was then used as a continuous trait in the genome-wide linkage analysis. LCA and GoM are both model-based approaches in which the optimum number of clusters was determined by likelihood ratio, Bayesian Information criteria (BIC) and Akaike information criteria (AIC). For Fanny, the number of clusters was set to 2, analogous to previous Fanny-based genetic studies (Kaabi and Elston 2003; Kaabi et al. 2006).

Phenotype data

Migraine data were obtained from extensive semi-structured telephone interviews as part of a study designed to assess physical, psychological and social manifestations of alcoholism and related disorders (Heath et al. 2001). The sample was unselected with regard to personal or family history of alcoholism or other psychiatric or medical disorders Mulder et al. (2003). The interviews were conducted during two periods of time: 1993–1995 and 1996–2000. The earlier interviews were administered to Australian twins listed with the volunteer-based Australian Twin Registry who were born between 1902 and 1964, whereas the second interviews were focused on twins born between 1964 and 1975.

Participants of both cohorts were first asked the screening question: "Do you have recurrent attacks of headaches?" If the participant screened positive, then he/she was asked a number of questions which were developed by an experienced migraine researcher based on the IHS diagnosis criteria (Table 4). A total of 13,062 individuals from 6,764 families participated in this study, with 2,716 MZ twin pairs (63.6% females and 36.4% males), 3,399 DZ twin pairs (34.52% female twins, 22.36% male twins and 43.13% mixed sex twins), 15 twins with unknown zygosity and 817 first degree family members, including both siblings and parents. The mean age of participants was 37.5 ± 11.3 and ages ranged from 23 to 90 years at the time of interview.

Although the wording of questions was identical for both cohorts, not all questions in Table 4 were included for the older cohort. The questions relating to having more than 5 migraine/episodes of headache during lifetime ("≥5 episodes"), average duration of migraine/episodes between 4 and 72 h ("4–72 h"), and pain associated with headache described as moderate to severe ("mod/severe") were not include in the questionnaire for the older cohort. We conducted separate analyses for older, younger and two cohorts combined data.

Models

Latent class analysis (LCA)

Latent class analysis is a multivariate technique which can be applied to clustering, regression and factor analysis. The classes are latent because they are not directly observed,

Table 4 The survey questions designed based on 1988 International Headache Society diagnostic criteria

Notation	Abbreviation	Descriptions
a	≥5 episode	Have at least 5 episode of headaches in your life time.
b	4–72 h	Average headache lasts between 4 and 72 h
c1	Unilateral	Headache often occurs at one side of head
c2	Pulsating	Headache pain can be described as throbbing, pulsating or pounding
c3a	Moderate/severe	Headache pain can be described between moderate and severe
c3b	Prohibitive	Headache pain prohibits daily activities
d1	Nausea/vomiting	Headache associated with vomiting or feeling nausea
d2a	Photophobia	Enhanced sensitivity to light
d2b	Phonophobia	Enhanced sensitivity to sounds
Aura	Aura	Have visual problems such as light shower, blurring, blind spot or double vision

but are identified based on a function of a set of observed variables. LCA was developed in the 1950s for dichotomous variables (Lazersfeld 1950); however, the full potential and practical application of LCA only became evident after the introduction of more general latent class analysis and a simpler method of obtaining maximum likelihood estimates of the parameters in the 1970s (Goodman 1974a, 1974b). The latter LCA is capable of dealing with both dichotomous and polytomous variables and more than one latent variable can be included in the model.

Suppose there are n individuals, J observed (manifest) variables and each variable j has L_j levels of response, $i = 1, 2, ...,n$, $j = 1, 2, ...,J$ and $l = 1, 2, ...,L_j$. Let y_{ijl} denote the binary response of the ith individual to symptom j with level l and Y_i is then the vector of subject i's response to all symptom questions. Assuming there are K latent classes within the latent variable, let λ_{kjl} denote the class conditional probability that an observation in class k produces the lth outcome on the jth variable; therefore, within each j, $\sum_l \lambda_{kjl} = 1$. In this paper, the data consist of binary responses, and thus L_j is two. Assuming local independence, the probability of a particular set of responses from an individual i in class k is:

$$f(Y_i|\lambda_k) = \prod_{j=1}^{J}\prod_{l=1}^{L_j}(\lambda_{kjl})^{y_{ijl}} \quad (1)$$

Let p_k denote the weight of latent component k. Then the joint distribution for all J variables under the latent class model is

$$Pr(Y_i|\lambda, p) = \sum_{k=1}^{K} p_k \prod_{j}^{J}\prod_{l}^{L_j}(\lambda_{kjl})^{y_{ijl}}$$

The LCA analyses were carried out using the poLCA (Linzer and Lewis 2007) package of R2.4.1 (R Development Core Team 2006). The parameters were estimated via the expectation–maximization (EM) algorithm (Dempster et al. 1977). The details of the EM algorithm for LCA are in Linzer and Lewis (2007). Unlike the other models described in this paper, the class membership probabilities are estimated post-hoc using Bayes' formula:

$$p_{ik} = Pr(k|Y_i) = \frac{p_k f\left(Y_i|\hat{\lambda}_k\right)}{\sum_k p_k f\left(Y_i|\hat{\lambda}_k\right)}, \quad (2)$$

where $\hat{\lambda}_k$ is an estimate of outcome probability conditioning on class k. Because the parameters are estimated using the EM algorithm, the latent class for the observations with missing value(s) can still be estimated. This is achieved by excluding cases with missing values when calculating Eq. 1 and the denominator of Eq. 2 (Lazersfeld 1950).

Grade of membership (GoM)

Grade of membership (GoM) also fits into the latent class framework. GoM was first developed by Woodbury et al. (1978) for expressing non-stochastic heterogeneity in a population by direct latent variable estimation. This method has been further developed by various researchers and is frequently applied in medical and genetic research (Erosheva 2002a).

Let $g_i = (g_{i1}, g_{i2},...,g_{iK})$ be the latent vector of grade membership scores for individual i having a partial membership of component k, where $g_{ik} \geq 0$ for each i and k and $\sum_{k=1}^{K} g_{ik} = 1$. The value g_{ik} can be interpreted as the intensity of membership in each component. Unlike LCA, the membership scores of individuals are estimated directly from data. Let λ_{kjl} denote the probability of positive response to level l of variable j for a complete membership of component k, $\lambda_{kjl} = Pr(x_{ijl} = 1|g_{ik} = 1)$ where $i = 1, 2,...,n$,

$j = 1, 2,...,J$ and $k = 1, 2,...,K$. Within each variable j, $\lambda_{kjl} \geq 0$ and, the sum of λ_{kjl} across all levels, is equal to one. The joint likelihood of GoM is

$$Pr(Y|\lambda, g) = \prod_{i=1}^{N} \prod_{j=1}^{J} \prod_{l=1}^{L_j} \left(\sum_{k} g_{ik} \lambda_{kjl} \right)^{y_{ijl}} \quad (3)$$

Equation 3 is maximized by iterative optimization with respect to one set of parameters while keeping the other set of parameters constant. This iterative procedure is referred to as the missing information principle. The details of the parameter estimation procedure are in Manton et al. (1994).

GoM can deal with missing values in two different ways, depending on the nature of the missing values. When the missing data are generated by a random mechanism which is independent of model parameters, missing data can be treated as unobserved and independent observations. In this case, y_{ijl} for a missing observation is set to be 0 for $l = 1, ...,L_j$ and is consequently excluded in the computation of the likelihood. When the missing data are due to a non-random process, such that certain items have a higher rate of missing data on a specific latent class, GoM deals with this problem by increasing the dimension of the measurement spaces by adding an extra category called "missing" for each variable in the model. In this study, in light of no information to the contrary, we assume the missing value is due to random causes.

The above models were tested using the Akaike information criterion (AIC, Akaike 1974), Bayesian information criterion (BIC, Schwarz 1978) and log-likelihood values for each value of K. AIC and BIC strike a balance between goodness of fit and model complexity, thus avoiding both over-fitting and under-fitting. Models with lower AIC and BIC values are preferred. Log-likelihood measures model fit but not complexity, and thus must be used cautiously to avoid over-fitting.

Phenotype conversion

In this study, the maximum number of components tested in the LCA and GoM analyses is 6 [max(K) = 6]. The optimum number of components for LCA is determined by the Bayesian information criteria (BIC) (Schwarz 1978) whereas the likelihood ratio test is used to determine the optimum number of components in GoM. Because both models yield only multinomial estimates, an intermediate step is added to obtain a continuous phenotypic measure. When the optimum value of K is 2, the membership score for the "affected" component (the component with more and stronger symptoms, such as p_{ik} = affected of LCA and g_{ik} = affected of GoM) is taken to be representative of the trait value. Currently, genome-wide linkage analysis is limited to either a continuous or a dichotomous trait value, and is not designed for multiple clusters. Therefore, in the past, when the optimum number of clusters in the model exceeded two, the phenotype was determined by a threshold value (Nyholt et al. 2004, 2005; Ligthart et al. 2006). To avoid the difficulty in determining an appropriate threshold, we implemented the following method to convert multinomial values to continuous values bounded between 0 and 1.

When the optimum number of components in a model exceeds 2, we used the following equation to estimate each individual trait value. Since this trait value aims to capture the presence of the symptom, we set l to 2:

$$\text{Phenotypic trait}_i = \sum_{k=1}^{k=K} \frac{\sum_{j=1}^{j=J} \lambda_{kj2}}{J} \times g_{ik}$$

where g_{ik} is membership score for individual i having partial membership of cluster k and J is the total number of manifest variables.

The use of a single, continuous-valued summary of phenotype such as this is not appropriate if two or more distinct disorders were producing the observed symptoms. We note that in the analysis of the migraine data, the clusters can be ordered sequentially such that the probability of experiencing each of the ten symptoms decreases monotonically. This is highly suggestive of a single underlying determinant of severity. The justification is less clear for the GoM model, because the clusters cannot be ordered in the same way. Nevertheless, the GoM clusters can be ordered such that the endorsement probabilities decrease monotonically for eight of the ten symptoms. Moreover, as we discuss below, there is reason to believe (on the basis of information criteria) that the LCA clustering is the more appropriate data model.

Grade of membership-Fanny

Unlike the two model-based approaches described above, Fanny forms clusters based on the dissimilarity between subjects, such that where subjects resemble each other they tend to be clustered into the same group. Dissimilarity between two objects can be calculated in various ways. Due to the type of variables in the migraine dataset, the dissimilarity matrix is calculated using a contingency table. Considering two objects, i and j, and the contingency table of i and j for variable p as shown in Table 5, the dissimilarity between i and j is estimated as

$$d(i,j) = \frac{b + c}{a + b + c + d}.$$

Let u_{ik} denote the strength of membership of object i to cluster $k, u_{ik} \geq 0, \sum_{k=1}^{K} u_{ik} = 1$. u_{ik} is analogus (but not

Table 5 The contingency table of object i and j

$i \setminus j$	1	0
1	a	b
0	c	d

equal) to g_{ik} and p_{ik} above. The objective of Fanny clustering is to iteratively minimize:

$$\sum_{k=1}^{K} \frac{\sum_{i,j=1}^{n} u_{ik}^2 u_{jk}^2 d(i,j)}{2\sum_{i=1}^{n} u_{jk}^2}.$$

Unlike LCA and GoM, Fanny does not provide a measure of how many clusters best fit the data; the user must choose the value of K. We therefore followed the approach utilized in previous Fanny-based genetic studies (Kaabi and Elston 2003; Kaabi et al. 2006) and fixed the number of clusters in the model to two. Whether this is appropriate or not would depend on the underlying architecture of the trait (symptomatology) under investigation. As a result, the phenotypic value of the individual subject was simply the score, u_{i2}, for the membership of the affected group. The Fanny algorithm procedure is implemented by the Fanny function of the contributed package cluster (Maechler and Hubert 2008) of the R (R Development Core Team 2006) statistical package.

Genetic data

The genotypic data are from a collection of four smaller genome-wide linkage scans performed for studies at the Queensland Institute of Medical Research (QIMR). Genotyping for four scans was undertaken at Gemini Genomics with 426 microsatellite markers, Sequana Therapeutic with 519 markers, the Center of Mammalian Genetics at the Marshfield Clinic Research Foundation with 776 markers and the University of Leiden with 435 markers. The recruitment of participants for genotyping was based on individuals involved in phenotype collection. The details of DNA collection, genotyping methods and data are provided in Zhu et al. (2004) and Cornes et al. (2005).

Graphic representation of relationships (GRR) (Abecasis et al. 2001) and RELPAIR (Epstein et al. 2000; Duren et al. 2003) were applied for the examination of the pedigree structure and identification of inconsistencies between the genotypic data and self-reported pedigree relationships. Potential pedigree misspecification, incorrect zygosity labelling of twins and potential sample mix-up were identified and investigated; the problematic individuals or families were removed from further analysis. The SIBPAIR program by Duffy (2002) was then implemented for identifying and cleaning the Mendelian inconsistencies in the genotype data.

After combining all four scans, there were 485 markers which were typed in two or more scans. Therefore to ensure the consistency of genotypic information for these 458 markers, the duplicated markers are included separately on the genetic map for the combined scan, which is separated by a small distance of 0.001cM. The consistency of the genotypes of these 458 markers was checked using various methods described in Cornes et al. (2005). Markers with genotypic data inconsistent between different genome scans were excluded and unlikely genotypes were identified by MERLIN (Abecasis et al. 2002) and omitted from further analysis. Potential map errors were identified by GENEHUNTER (Kruglyak et al. 1996) and MENDEL (Lange et al. 1988). Map positions were in Kosambi cM, which is estimated using locally weighted linear regression from the National Center for Biotechnology Information (NCBI) Build 34.3 physical map positions, as well as published deCODE and Marshfield genetic map positions (Kong et al. 2004). Where the results suggested inconsistencies between genetic map distance and recombination fraction, the primer sequences for all markers in the region were BLASTed against the entire human genome sequence (http://www.ensembl.org, NCBI build 34.3). The genetic map was then revised to include the updated physical positions of all markers in the problematic regions. The revised map and the original genotype data were cleaned of unlikely genotypes using MERLIN and map errors were resolved using GENEHUNTER.

The final cleaned data contains 1,770 unique markers. The main intermarker distance for all sib-pairs in the samples was 7.1cM, calculated for each sib-pair and analyzed across all sib-pairs. The combined genome scan included 4,148 individuals from 919 families, which included 143 MZ and 776 DZ twin pairs.

Heritability

Heritability of the continuous phenotype values was estimated with the ACE model. The ACE model assumes the phenotype variation is due to the additive genetic effect (A), shared environment effect (C) and random environment effect (E). The heritability is then the proportion of the total variance which is due to the additive genetic effect. The analysis was carried out using Mx (Neale et al. 1997) which performs maximum likelihood estimation of the variance components.

Linkage analysis

A non-parametric quantitative trait linkage analysis was carried out using Merlin-qtl, developed under the general framework of Kong and Cox (1997) and Whittemore and Halpern (1994). The membership score of the three models

(g_{ik} of LCA and GoM and u_{ik} of Fanny) was treated as a quantitative trait.

Results

The results of clustering and linkage analyses performed separately for the older and younger cohorts lack the power to identify any significant loci. Moreover, the analysis of the older cohort itself is not representative of the true migraine population due to lack of three symptom responses. However, by combining two cohorts, we obtained a representative sample and power to identify disorder-related loci, hence we restrict our subsequent results to the combined data set.

Table 6 provides goodness of fit statistics for the choice of k in the two model based approaches, LCA and GoM. For LCA, there is little improvement in AIC or BIC as K increases above four, where there is a local minimum in BIC (Table 6). We therefore selected $K = 4$ as the best compromise between model fit and complexity. For GoM, both AIC and BIC indicate that the best model has $K = 2$, but even this best-scoring GoM model is substantially worse than any of the LCA models. The reason for this is that although GoM models have better fit (that is, higher log-likelihood), they achieve this at the cost of including additional parameters, namely the membership scores g_{ik}. In light of this, we based goodness of fit assessment on the log-likelihood ratios and noting that the largest reductions in log-likelihood occur as K increases to four, we again chose the four clusters GoM model.

Even though four clusters were chosen for both GoM and LCA, the characteristics of the clusters differ between these phenotyping approaches. Figure 1 shows the characteristics of each LCA cluster. Each bar shows the probability of having the symptom, given a full membership to cluster k.

Table 6 The log-likelihood value, AIC and BIC values of LCA and GoM models with different number of clusters

Model	Number of cluster (K)	Log-likelihood	AIC	BIC
LCA	2	−38752.642	77549.28	77713.79
	3	−36677.701	73423.4	73677.63
	4	−36456.261	73004.52	73348.49
	5	−36401.638	72948.79	73382.48
	6	−36333.290	72806.58	73330
GoM	2	−28616.94	109561.9	305202
	3	−22696.07	123884.1	417344.6
	4	−20978.36	146612.7	527892.4
	5	−20322.00	171464.0	660564.8
	6	−18838.39	194660.8	781581.8

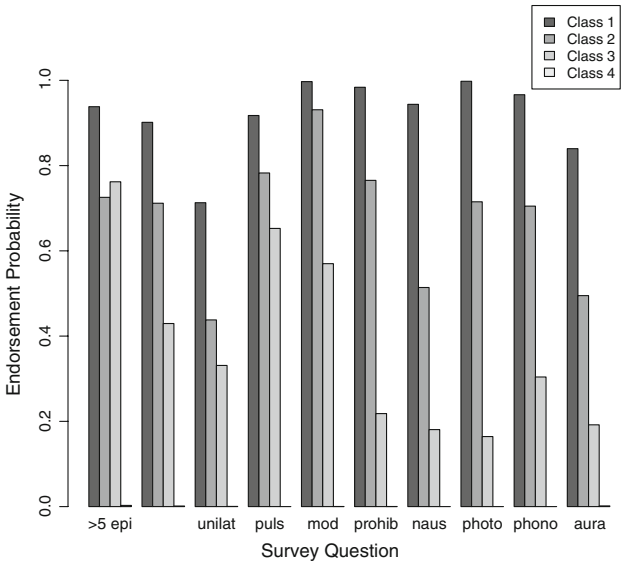

Fig. 1 The characteristics of the four clusters under LCA $K = 4$ model. X-axis corresponds to the items listed in Table 4 and the y-axis is the probability of displaying the symptom given full membership to cluster k

For instance, the probability of having "aura" for a member in cluster 1 is 0.90. There is a progressive reduction of endorsement probability for all symptoms when cluster 2 is compared to cluster 1, when cluster 3 is compared to cluster 2 and when cluster 4 is compared to cluster 3. The only departure from this pattern is the slight increase in the probability of a positive response to the question "have you had more than 5 episodes of headaches in your life time?" when cluster 3 is compared to cluster 2. The clusters are thus in a natural order, suggesting, as mentioned earlier, that migraine phenotypes can be organised on a linear scale of severity.

This linear pattern is not apparent for the GoM clusters. It is apparent that cluster 1 has the highest endorsement probabilities for all symptoms and cluster 4 has the lowest. However, although cluster 2 has equal or higher endorsement probabilities than cluster 3 for most symptoms, this situation is reversed for the symptoms " ≥5 episodes" and "moderate/severe" (Fig. 2).

The profile plot showing the characteristics of the Fanny clusters is depicted in Fig. 3. There is a large difference in the endorsement probabilities of the two clusters, and more than 55% of individuals in cluster 2 have all symptoms listed in Table 4. Individuals in cluster 2 are not exempt from all symptoms; a small proportion in this cluster had the first five symptoms of Table 4 during their headache episode. Since there are only two clusters in this analysis, cluster 1 can be referred to as the "Affected" class and cluster 2 as the "Unaffected" class.

Of the total 13,062 individuals, 14% were assigned to cluster 1, 21% to cluster 2, and 10 and 55% were in cluster

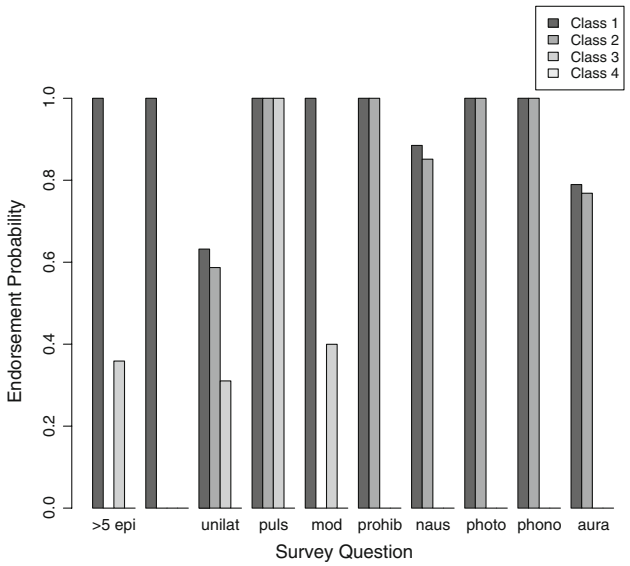

Fig. 2 The characteristics of the four clusters under GoM $K = 4$ model. *X*-axis corresponds to the items listed in Table 4 and the *y*-axis is the probability of displaying the symptom given full membership to cluster *k*

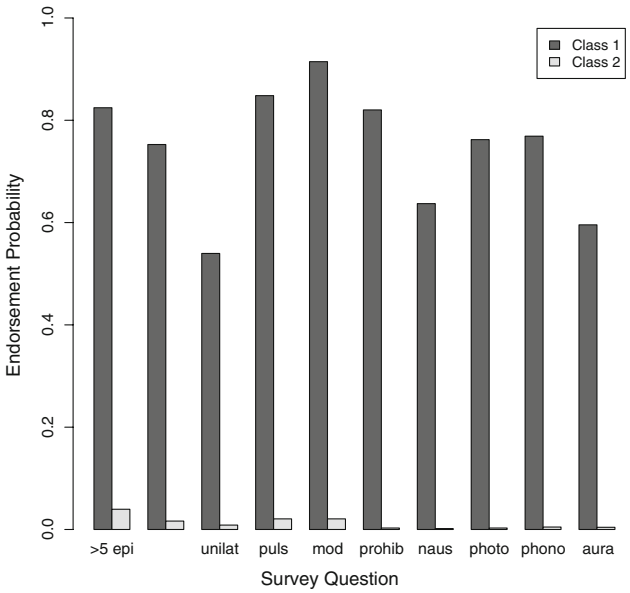

Fig. 3 The characteristics of the four clusters under Fanny $K = 2$ model. *X*-axis corresponds to the items listed in Table 4 and the *y*-axis is the proportion of individuals having the symptom given cluster *k*

3 and 4, respectively, according to LCA (Table 7). In contrast to LCA, a slightly higher proportion of the population were classified into the two extreme clusters of GoM with 22% falling into cluster 1 and 60% into cluster 4. Under the Fanny clustering method, around 40% of the population are classified into cluster 1 and 60% are in cluster 2 (Table 7).

Table 7 The weight of each cluster under different phenotyping analysis

Model	No. of clusters	Class 1 (Affected)	Class 2	Class 3	Class 4 (less affected)
LCA	4	0.136	0.206	0.103	0.554
GoM	4	0.215	0.076	0.105	0.604
Fanny	2	0.405	0.590	–	–

For LCA and GoM, the optimum number of clusters is 4 whereas the cluster size is 2 for Fanny

– Not applicable

After phenotype conversion, all three models agreed that a large proportion of the subjects in this study have a very small probability of having had migrainous headaches (Fig. 4). However, we observed some variations in the tail end of the histograms. According to GoM, there is an even distribution in the individuals with scores between 0 and 1, with a slightly higher proportion having scores closer to 1. This is different from the results obtained using Fanny and LCA, in which only a very small number of people had a phenotypic score between 0 and 0.4. However, unlike the tail end of the Fanny histogram which shows a slight increase in score distribution, the LCA histogram shows small peaks at 0.5 and 0.7. The maximum trait scores estimated in LCA and GoM approach 1, whereas the maximum trait score using Fanny is 0.86.

At the individual level, LCA and Fanny gave similar phenotypic estimates. Figure 5 contains scatter plots showing the predicted scores of individuals under the different methods. LCA and Fanny show very similar predicted scores when the score is larger than 0.4. Although Fanny tends to give higher phenotypic scores to individuals with a score lower than 0.4, generally there is a strong correlation between LCA and Fanny phenotypic scores (correlation = 0.99). In contrast, although the correlation is still high (correlation = 0.85), there is a notable discrepancy between LCA and GoM predicted scores. This is also observed in the comparison of phenotypic scores obtained using the Fanny and GoM approaches.

Table 8 contains the heritability estimates when using the phenotypic scores of the three models where A indicates the variation due to genetic variation, C is the variation due to the shared environmental effects and E is the effect due to non-shared environmental effects. The range of heritability is between 0.36 and 0.46. The highest heritability occurs when using the phenotype derived from the GoM model, which is 0.46 with a 95% confidence interval of 0.43–0.49. This indicates, if the assumptions for the ACE model hold, that 46% of total variation is due to genetic variation, none of the variation is due to shared environment effects and nearly 54% is due to the random environmental effects.

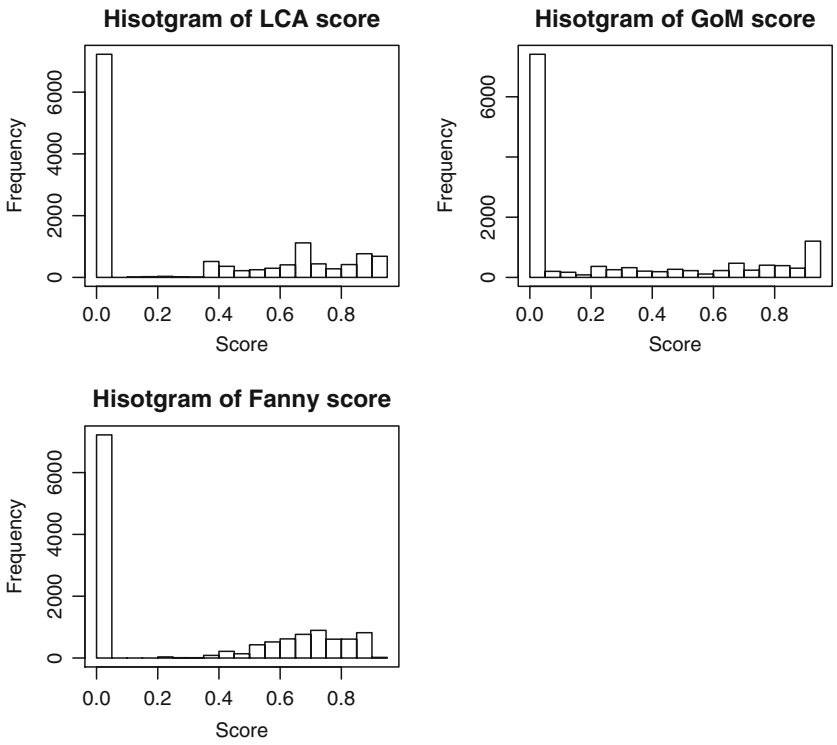

Fig. 4 Histogram showing the distribution of the phenotypic scores estimated under LCA, GoM and Fanny. A score of 0 indicates not having migrainous headache and a score of 1 indicates having strong migrainous headache

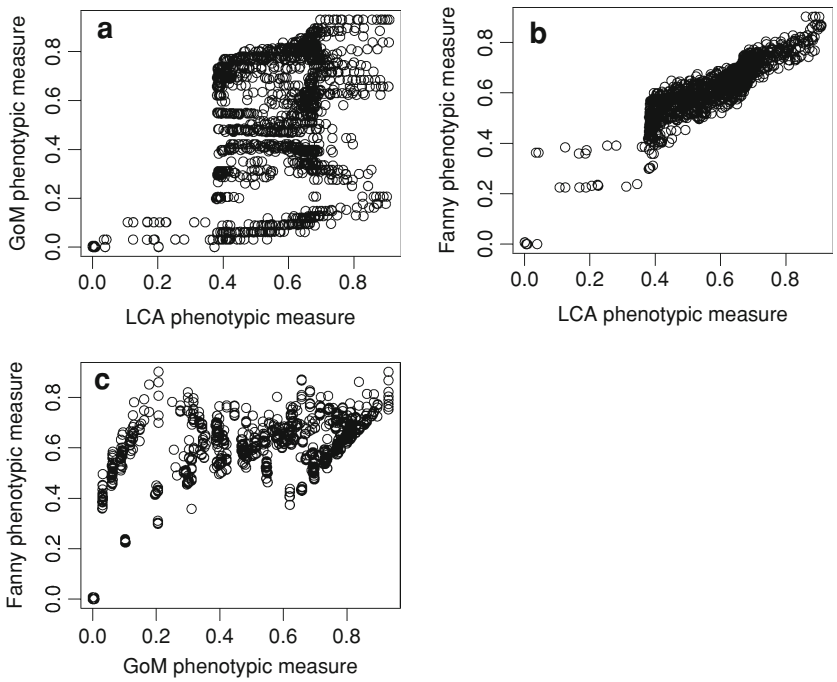

Fig. 5 Scatter plots showing the relationship between phenotypic scores estimated under different methods. The *top left* plot is the estimated phenotypic score from LCA vs GOM. The *top-right hand* plot is the comparison in estimated trait between LCA and Fanny approaches; the *bottom plot* is the comparison of estimated trait between GoM and Fanny approaches

The heritability estimates obtained using LCA and Fanny phenotypes are close: respectively, 37 and 36%. The variation due to shared environmental effects is consistent between these two approaches, and is in line with that obtained for the GoM approach. The non-shared environmental effects for these two approaches are 63 and 64%.

Merlin-qtl multipoint LOD scores using the three different phenotypic measures were calculated at 1-cM increments; see Fig. 6. The black solid line is the LOD score corresponding to the LCA phenotype; the red dashed line corresponds to GoM and the green dotted line corresponds to Fanny. The LOD scores based on LCA and

Table 8 The migrainous headache heritability estimates from the ACE model, where A is the variation due to genetic variation and C is the variability due to environmental effect

Model	BIC	Components	Mean	Lower CI	Upper CI
LCA	−48352.60	A	0.3710	0.3365	0.4007
		C	0.0000	0.0000	0.0000
		E	0.6290	0.5993	0.6569
GoM	−48429.35	A	0.4625	0.4308	0.4905
		C	0.0000	0.0000	0.0000
		E	0.5375	0.5095	0.5665
Fanny	−48079.38	A	0.3592	0.3266	0.3877
		C	0.0000	0.0000	0.0000
		E	0.6408	0.6104	0.6720

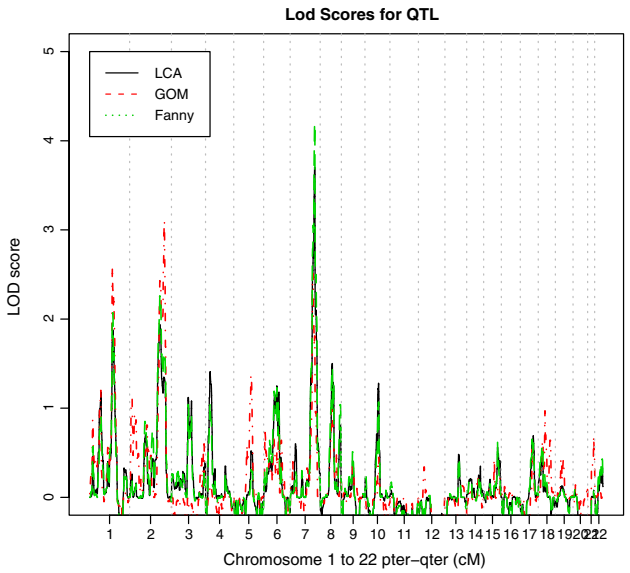

Fig. 6 Results of MERLIN-qtl genomewide linkage analysis using traits derived from different statistical clustering methods. The *solid black line* is the LOD score of traits derived from LCA, *red dashed line* is the LOD score of trait from GoM and *green dotted line* is LOD score of Fanny traits. The *dotted vertical lines* indicate the boundaries between chromosomes

Fanny show very similar patterns with several regions on chromosome 7 having LOD scores over 3. The highest LOD scores are on chromosome 7 at the 136 cM region (LCA LOD=3.7; Fanny LOD=4.12) followed by chromosome 7 at the 133 cM region. (LCA LOD = 3.28, Fanny LOD = 3.47). The third highest LOD score is also found in chromosome 7 at 127cM (LCA LOD = 2.72, Fanny LCA = 3.05). Although the LOD score signals are not as high as in chromosome 7, the genomewide linkage analysis shows possible evidence of linkage on chromosomes 2 and 1 in LCA and Fanny traits. Markers D28364 G, GATA194A05 and D2S1391, which are between 187 and 188 cM of chromosome 2, have a LOD score of 1.89 based on the LCA traits and 2.25 for the Fanny traits; and marker ATA73A08 (156cM) on chromosome 1 shows a small peak.

In contrast, the LOD scores based on the GoM phenotypes show a very different pattern. The highest LOD score of the GOM trait is on Chromosome 2 between 210 cM (LOD = 3.10); followed by chromosome 2 at the 206 cM region (LOD = 2.81). Some signals are detected on chromosome 1 and 7; marker AGAT119 M (153 cM) on chromosome 1 has a LOD score of 2.59 and marker ATA55A05 M (127cM) on chromosome 7 has a LOD score of 2.51.

Discussion

Genetic research of diseases with a complex etiology firstly requires the identification of phenotypes which capture the underlying phenotypic and genetic variance. In this study, the aim was to investigate the effects of different clustering methods on the output of genetic analyses using a previously described (Nyholt et al. 2005) and subsequently updated migraine dataset. We tested three commonly used statistical clustering phenotyping methods: LCA, GoM and Fanny. Of these, the first two are model-based approaches, whereas Fanny is based on partitioning of a dissimilarity matrix. Our results show that with the same symptom response data, different phenotype clusters are derived and as a consequence different genetic loci are implicated via linkage.

The heritability estimated here with three different migraine phenotypic traits is within the range of previously published findings (Mulder et al. 2003). Mulder et al. (2003) show that the heritability of MA and MO varies for different populations. For the Australian population, previously published results indicate the heritability varies as different phenotyping methods are applied (Nyholt et al. 2004); this is supported by our findings. The ACE model fitting indicated the greater genetic contribution to migraine using GoM, followed by LCA and Fanny, which are 46, 37 and 36%, respectively. Some of these estimates are higher than the heritability for the IHS criteria defined phenotype published in Nyholt et al. (2004, 2005). We also noted that differences in heritability can occur within a model. For instance, using the same LCA model, the h^2 of the converted continuous trait is slightly lower than the h^2 of the dichotomous trait in Nyholt et al. (2004, 2005). We failed to identify the shared environmental effects for these phenotypic traits, as also occurred in Nyholt et al. (2004). Nyholt et al. (2004) found that when additive genetic effects are present, the power to detect the shared environmental effects is low.

The difference between the continuous trait values derived from the LCA and GoM models is mainly due to

the different clustering structure. Although the number of clusters in both models are the same, the characteristics of clusters are very different. The clusters of the GoM model differ in symptom composition but the clusters of the LCA model are different in the probability of having all ten symptoms.

The two model-based clustering methods implicated different genetic loci. However, based on the GoM phenotype, linkage was obtained to a locus near marker D2S2944 on chromosome 2 and to loci on chromosomes 1 and 7. Conversely, the two most unlike clustering methods, LCA and Fanny, not only produced linkage at the same positions but also gave the same ranking to those positions. The linkage analysis of LCA- and Fanny-based traits had highest LOD scores at Chr7q33 and Chr7q32.3 regions, respectively.

Although the markers with the highest LOD scores in the LCA and Fanny phenotype analyses are not implicated in the GoM linkage results, the genetic analysis of GoM produced linkage to other possible markers on chromosome 1 and 7. Marker AGAT119M of chromosome 7 has the fifth highest LOD score for the GoM trait, and the third highest LOD score ranking of the LCA and Fanny traits. In contrast, although linkage analysis of LCA and Fanny traits did not provide strong evidence for linkage to marker AGAT119M on chromosome 1 (LOD scores less than 2), there is still some evidence of linkage.

Although the LOD scores for some loci are less than 3, our analysis was able to replicate some previously identified regions. The small peak on chromosome 1 of LCA and Fanny traits is within 2cM of the familial hemiplegic migraine (FHM) type 2 ATP1A2 gene (De Fusco et al. 2003; Vanmolkot et al. 2003). The small peak in chromosome 2 is also within a small distance of the SCN1A FHM3 gene found by another study (Dichgans et al. 2005). Another important marker is GGAT1A4, which is located on the chr 10q22.3–10q23.1 region. Our genome-wide linkage results indicated a suggestive linkage in this region. This is encouraging because the same region has been identified previously by Anttila et al. (2008, 2006) and Nyholt et al. (2005). Unlike much other research, Anttila et al. (2008) adopted three different methods to phenotype the migraine patients of the Australian and Finnish populations; this includes the less stringent form of IHS defined MA, LCA and trait-component analysis. Note the phenotypic traits derived from their LCA is calculated using a different algorithm from the one used here and Anttila et al. (2008) implement the same algorithm as the one described in Nyholt et al. (2004). We will later explain the difference between these two approaches and discuss the effects of these algorithms on linkage analysis. Previously detected loci, chr 6p12.2–p21.1 and 8q21 (Nyholt et al. 2005), are also detected here with suggestive linkage when the trait values are derived from LCA and Fanny.

Some previously identified loci were not detected here; this includes 4q21 (Björnsson et al. 2003), 4q24 (Wessman et al. 2002; Anttila et al. 2006; Lea et al. 2005), 4q21–q31 (Anttila et al. 2006), 5q21 Nyholt et al. (2005), 8q21 (Anttila et al. 2008; Nyholt et al. 2005), 14q21–q23 (Soragna et al. 2003; Anttila et al. 2008), 15q11–q13 (Anttila et al. 2006), 17q13 (Anttila et al. 2006), 18q12 (Björnsson et al. 2003; Wessman et al. 2002; Anttila et al. 2008). Here are some possible causes of this difference. Firstly, the common form of migraine, according to IHS cirteria, is an ensemble of multiple symptoms; each symptom may be caused by specific loci and these loci contribute to susceptibility to migraine (Nyholt et al. 2005; Anttila et al. 2006; Lea et al. 2005). For the formation of common migraine, genes may need to act synergistically. One drawback of single-locus linkage analysis is that it is not able to detect epistasis effects, which commonly present in a complex disease. Therefore, the development of genome wide association studies in conjunction with statistical tools for detecting epistasis effects is more suitable for detecting the genetic architecture of migraine.

Another possible cause for not replicating previously detected loci is the variation of phenotyping methods adopted in other studies. Our results indicate that different phenotyping methods can result in different loci being identified in linkage analysis; hence it is not surprising that some previously prominent genes go undetected here. We do not advocate our findings as superior to others, or vice versa, but they do demonstrate the need to base linkage analysis on different trait values derived from various methods to ensure the validity of the conclusion. This is especially true for diseases with complex etiology.

Differences in the results of genetic analyses can occur not only between models, but also within a model. Nyholt et al. (2004, 2005) applied LCA to migraine survey data and identified four subgroups of migraine/severe headaches. Individuals classified into clusters 2 and 3 were treated as "affected" and given a trait value of 1 and conversely individuals in the other two clusters were given a trait value of 0. The authors then conducted a regression using MERLIN and found the highest LOD scores on chromosomes 5, 10, 8, 1 and 6. Although the current results cannot be readily compared to those present in Nyholt et al. (2005), due to differences in available phenotype data and modelling approach, we replicated their procedure and generally we found lower LOD scores but in similar positions to those identified by Nyholt et al. (2005). The main difference between the approaches used by Nyholt et al. (2005) and those in the current paper is that the former employed discrete cluster membership as an

"affection" trait, whereas the current results utilized a continuous phenotypic score related to cluster membership.

To investigate further the effect of different clustering approaches on within-model effects, we separately tested the LCA and GoM models with predefined values of K. When $K = 2$, the results of the genetic analysis based on both the LCA and GoM are different from those obtained when $K = 4$. Within a GoM phenotyping analysis, when K is 2, the highest LOD score is 2.29 at D1S484 on chromosome 2, which is 53 cM from the loci identified using the optimum GoM model. The within-model effect is more apparent for the LCA phenotypes, where not only the linkage position changed, but the highest LOD score reduced from 3.70 to 2.03. This demonstrates the influence of the number of clusters on the model-based clustering approaches.

The likelihood ratio test statistics and BIC used in the present analysis for model selection are common parsimony criteria but are not ideal for mixture models (Marin et al. 2005). More sophisticated methods, such as bootstrapping (McLachlan et al. 1999) or reversible jump Markov chain Monte Carlo methods (RJMCMC) (Richardson and Green 1997), may be more effective in searching for the optimum number of clusters in a finite sample space. The work by Berkhof et al. (2003) provides a framework for using Bayes factors for component selection in mixture models.

Despite the fact that LCA and GoM are both forms of mixture models, they are quite different in practice. In GoM, the membership scores of individuals are estimated as model parameters, so the number of parameters in the model increases dramatically with the sample size. The increase in number of parameters not only slows down the computation of the model, but it also has an effect on the determination of the optimum number of components, where the criteria for model selection are based on a parsimony measure.

Another drawback of GoM, which is also shared by LCA, is in the algorithm for parameter estimation. Both of these methods are implemented using an iterative algorithm, such as EM, to find maximum likelihood estimates. These procedures may only find the local maximum as the model becomes complex (Linzer and Lewis 2007). Therefore, to ensure the achievement of a global maximum, re-estimation of the model parameters with multiple starting points is recommended. As is common in such cases, it is difficult to provide guidance as to how many starting points should be used, but one rule of thumb is to repeat the optimization until each observed local maximum is attained from more than one starting point.

The large number of parameters involved in the GoM model can also result in instability of the estimation of membership score, g_{ik}. Manton et al. (1994) has suggest various modifications to improve consistency: in particular, by assuming g_{ik} for individual i is a realization of a random vector, with a distribution function.

Although the Fanny algorithm is relatively simpler and computationally easier, there are some limitations associated with this approach. Firstly, the Fanny algorithm clusters data without taking into account any structure in the data. It is therefore essential to have two extreme response patterns in the data, ideally individuals with all symptoms, and individuals without all symptoms with heavy weights on both patterns.

Clustering using the Fanny algorithm is highly dependent on the dataset and consequently the clustering structure often changes when extra data are included in the analysis. Unless the sample is representative of the population, the phenotypic measures determined from a small sample may be biased. Another limitation of the Fanny algorithm is that as sample size and the number of questions increases, the computational requirements for the dissimilarity matrices also increase.

Of all three models, LCA is most computationally efficient, but it is not fully exempt from the effects of increasing parameter dimension. Computational time also increases rapidly with the number of latent classes (K), manifest variables (J) and levels within each manifest variable (L_j). When the number of parameters exceeds the number of samples, or one fewer than the total number of cells in the cross-classification table of manifest variables, the LCA will not be identifiable (Linzer and Lewis 2007).

This study is based on the assumption that the migrainous population is composed of multiple subgroups. But it remains uncertain that the population that suffers from migrainous headaches is unidimensional. Therefore, models such as latent trait analysis may exhibit better performance than any clustering based statistical methods.

In this paper, we adopted the somewhat innovative practice of converting cluster memberships to continuous phenotype scores. We regard this practice as preferable to the arbitrary imposition of a threshold, which effectively separates individuals into cases and controls. However, we urge caution in the use and interpretation of such phenotype scores. In particular, the practice assumes that the disease can be satisfactorily modeled as the result of a single, unidimensional, continuous determinant of severity. One should therefore investigate whether the clusters can be placed in a natural order of monotonically decreasing severity, as we have done here. We suggest further research into the relative merits of using continuous phenotype scores as opposed to thresholds.

In conclusion, different phenotyping methods have different properties; not knowing the true phenotypic structure of the population, phenotyping methods can therefore only

provide approximations to the trait. To minimise the impact of phenotypic uncertainties, we suggest the following alternative approaches:

1. *Phenotype integration* Run multiple phenotyping methods and integrate the results of different methods to produce a single phenotype. Then perform linkage analysis on this integrated phenotype.
2. *Eliminate ambiguous cases* Eliminate cases with phenotypes that differ for different phenotyping methods, thus limiting subsequent analysis to those individuals for which all methods produce essentially the same classification.
3. *Multiple linkage analysis* Run multiple linkage analysis on the phenotypic classifications derived from different models, using different clustering techniques and different numbers of classes. Then combine the results of these multiple analyses with a voting mechanism.

Such approaches may facilitate more stable estimation of genetic linkage for diseases with complex etiology. We recommend further research into the relative success of such approaches.

Acknowledgments This study is funded by National Health and Medical Research Council capacity-building grant (grant number 389892) and Professor Peter Visscher for constructive advice. The authors would also like to thank Dr. Elizabeth Corder and Dr Elena Erosheva for her help on Grade-Of-Membership. We would also like to thank Christopher Oldmeadow for his assistance with the computer programs.

References

Abecasis GR, Cherny SS, Cookson WO, Cardon LR (2002) Merlin-rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 30:97–101

Abecasis GR, Cherny SS, Cookson WOC, Cardon LR (2001) GRR: graphical representation of relationship errors, vol 17. Oxford University Press, New York

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Automat Contr 19(6):716–723

Anttila V, Kallela M, Oswell G, Kaunisto MA, Nyholt DR, Hamalainen E, Havanka H, Ilmavirta M, Terwilliger J, Sobel E (2006) Trait components provide tools to dissect the genetic susceptibility of migraine. Am J Hum Genet 79(1):85–99

Anttila V, Nyholt DR, Kallela M, Artto V, Vepsäläinen S, Jakkula E, Wennerström A, Tikka-Kleemola P , Kaunisto MA, Hämäläinen E (2008) Consistently replicating locus linked to migraine on 10q22–q23. Am J Hum Genet 82(5):1051–1063

Berkhof J, van Mechelen I, Gelman A (2003) A bayesian approach to the selection and testing of mixture models. Stat Sinica 13:423–442

Björnsson A, Gudmundsson G, Gudfinnsson E, Hrafnsdóttir M, Benedikz J, Skúladttir S, Kristjänsson K, Frigge ML, Kong A, Stefánsson K, Gulcher JR (2003) Localization of a gene for migraine without aura to chromosome 4q21. Am J Hum Genet 73(5):986–993

Cader ZM, Noble-Topham S, Dyment DA, Cherny SS, Brown JD, Rice GPA, Ebers GC (2003) Significant linkage to migraine with aura on chromosome 11q24. Hum Mol Genet 12(19):2511–2517

Carlsson A, Forsgren L, Nylander PO, Hellman U, Forsman-Semb K, Holmgren G, Holmberg D, Holmberg M (2002) Identification of a susceptibility locus for migraine with and without aura on 6p12.2–p21.1. Neurology 59(11):1804–1807

Cassidy F, Pieper CF, Carroll BJ (2001) Subtypes of mania determined by grade of membership analysis. Neuropsychopharmacol 25(3):373–83

Corder EH, Woodbury MA (1993) Genetic heterogeneity in alzheimer's disease: a grade of membership analysis. Genet Epidemiol 10:495–499

Cornes BK, Medland SE, Ferreira MAR, Morley KI, Duffy DL, Heijmans BT, Montgomery GW, Martin NG (2005) Sex-limited genome-wide linkage scan for body mass index in an unselected sample of 933 australian twin families. Twin Res Hum Genet 8(6):616–632

De Fusco M, Marconi R, Silvestri L, Atorino L, Rampoldi L, Morgante L, Ballabio A, Aridon P, Casari G (2003) Haploinsufficiency of atp1a2 encoding the na+/k+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. Nat Genet 33(2):192–6

Dempster AP, Laird NM, Rubin DB (1977) Maximum likelihood from incomplete data via the em algorithm. J Roy Stat Soc B 39(1):1–38

Dichgans M, Freilinger T, Eckstein G, Babini E, Lorenz-Depiereux B, Biskup S, Ferrari MD, Herzog J, van den Maagdenberg A, Pusch M (2005) Mutation in the neuronal voltage-gated sodium channel scn1a in familial hemiplegic migraine. Lancet 366(9483):371–377

Duffy DL (2002) Sib-pair version 0.99. 9. Queensland Institute of Medical Research, Brisbane, Australia

Duren WL, Epstein MP, Li M, Boehnke M (2003) Relpair: A program that infers the relationships of pairs of individuals based on marker data

Epstein MP, Duren WL, Boehnke M (2000) Improved inference of relationship for pairs of individuals. Am J Hum Genet 67(5):1219–1231

Erosheva EA (2002a) Grade of membership and latent structure models with application to disability survey data. PhD, Carnegie Mellon University

Erosheva EA (2002b) Partial membership models with application to disability survey data. In: Bozdogan H (ed) Statistical data mining and knowledge discovery. Chapman and Hall/CRC, Boca Raton, pp. 117–133

Erosheva EA (2005) Comparing latent structures of the grade of membership, rasch, and latent class models. Psychometrika 70(4):619

Fillenbaum GG (1998) Typology of alzheimer's disease: findings from cerad data. Aging Ment Health 2(2):105–127

Goodman LA (1974a) The analysis of systems of qualitative variables when some of the variables are unobservable. part ia modified latent structure approach. Am J Sociol 79(5):1179

Goodman LA (1974b) Exploratory latent structure analysis using both identifiable and unidentifiable models. Biometrika 61(2):215–231

Hallmayer JF, Jablensky A, Michie P, Woodbury M, Salmon B, Combrinck J, Wichmann H, Rock D, Ercole MD, Howell S, Dragovic M, Kent A (2003) Linkage analysis of candidate regions using a composite neurocognitive phenotype correlated with schizophrenia. Mol Psychiatr 8(5):511

Headache Classification Committee of the International Headache Society (1988) Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. Cephalagia 8:1–96

Heath AC, Howells W, Kirk KM, Madden PA, Bucholz KK, Nelson EC, Slutske WS, Statham DJ, Martin NG (2001) Predictors of non-response to a questionnaire survey of a volunteer twin panel: findings from the australian 1989 twin cohort. Twin Res 4(2):73–80

Jablensky A (2006) Subtyping schizophrenia: implications for genetic research. Mol Psychiatr 11:815–836

Kaabi B, Elston RC (2003) New multivariate test for linkage, with application to pleiotropy: Fuzzy haseman-elston. Genet Epidemiol 24(4):253–264

Kaabi B, Gelernter J, Woods SW, Goddard A, Page GP, Elston RC (2006) Genome scan for loci predisposing to anxiety disorders using a novel multivariate approach: Strong evidence for a chromosome 4 risk locus. Am J Hum Genet 78:543

Kaufman L, Rousseeuw PJ (1990) Finding groups in data: an introduction to cluster analysis. Wiley series in probability and mathematical statistics. Applied probability and statistics. Wiley, New York

Kong A, Cox NJ (1997) Allele-sharing models: Lod scores and accurate linkage tests. Am J Hum Genet 61(5):1179–1188

Kong X, Murphy K, Raj T, He C, White PS, Matise TC (2004) A combined linkage-physical map of the human genome. Am J Hum Genet 75(6):1143–8

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58(6):1347–1363

Lange K, Weeks D, Boehnke M (1988) Programs for pedigree analysis: Mendel, fisher, and dgene. Genet Epidemiol 5(6):471–2

Lazersfeld PF (1950) The logical and mathematical foundations of latent structure analysis. Princeton University Press, Princeton

Lea RA, Nyholt DR, Curtain RP, Ovcaric M, Sciascia R, Bellis C, MacMillan J, Quinlan S, Gibson RA, McCarthy LC (2005) A genome-wide scan provides evidence for loci influencing a severe heritable form of common migraine. Neurogenetics 6(2):67–72

Ligthart L, Boomsma DI, Martin NG, Stubbe JH, Nyholt DR (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(1):54–63

Ligthart L, Nyholt DR, Hottenga JJ, Distel MA, Willemsen G, Boomsma DI (2008) A genome-wide linkage scan provides evidence for both new and previously reported loci influencing common migraine. Am J Med Genet B Neuropsychiatr Genet

Linzer DA, Lewis J (2007) polca: Polytomous variable latent class analysis

Maechler MM, Hubert M (2008) The cluster package

Manton KG, Woodbury MA, Tolley HD (1994) Statistical Applications Using Fuzzy Sets. Wiley

Manton KG, Xiliang G, Hai H, Kovtun M (2004) Fuzzy set analyses of genetic determinants of health and disability status. Stat Methods Med Res 13(5):395–408

Marin JM, Mengersen K, Robert CP (2005) Bayesian modelling and inference on mixtures of distributions. Handbook of Statistics, vol 25 pp 459–507

McCutcheon AL (1987) Latent Class Analysis. Quantitative Applications in the Social Science. Sage Publications, Newbury Park

McLachlan GJ, Peel D, Basford KE, Adams P (1999) The emmix software for the fitting of mixtures of normal and t-components. J Stat Softw 4(2):

Mulder EJ, Van Baal C, Gaist D, Kallela M, Kaprio J, Svensson DA, Nyholt DR, Martin NG, MacGregor AJ, Cherkas LF (2003) Genetic and environmental influences on migraine: a twin study across six countries. Twin Res 6(5):422–31

Neale MC, V. I. for Psychiatric, Behavioral G, P. Department of, and V. Medical College of (1997) MX: Statistical Modeling. Department of Psychiatry, Medical College of Virginia

Nyholt DR, Gillespie NG, Heath AC, Merikangas KR, Duffy DL, Matrin NG (2004) Latent class and genetic analysis does not support migraine with aura and migraine without aura as separate entities. Genet Epidemiol 26:231–244

Nyholt DR, Morley KI, Ferreira MAR, Medland SE, Boomsma DI, Heath AC, Merikangas KR, Montgomery GW, Matrin NG (2005) Genomewide significant linkage to migrainous headache on chromosome 5q21. Am J Hum Genet 77:500–512

Olesen J, Steiner TJ (2004) The international classification of headache disorders, 2nd edn (icdh-ii). Brit Med J 75(6):808

Potthoff RF, Manton KG, Woodbury MA (2000) Dirichlet generalizations of latent-class models. J Classif 17(2):315–353

R Development Core Team (2006) R 2.4.1 a language and development

Richardson S, Green PJ (1997) On bayesian analysis of mixtures with an unknown number of components. J Roy Stat Soc B Met 59(4):731–792

Schwarz G (1978) Estimating the dimension of a model. Ann Stat 6(2):461–464

Silberstein S, Olesen J, Bousser MG, Diener HC, Dodick D, First M, Goadsby P, Gobel H, Lainez M, Lance J (2005) The international classification of headache disorders, (ichd-ii)-revision of criteria for 8.2 medication-overuse headache. Cephalalgia 25(6):460–465

Soragna D, Vettori A, Carraro G, Marchioni E, Vazza G, Bellini S, Tupler R, Savoldi F, Mostacciuolo ML (2003) A locus for migraine without aura maps on chromosome 14q21.2–q22.3. Am J Hum Genet 72(1):161

Svensson DA, Larsson B, Waldenlind E, Pedersen NL (2003) Shared rearing environment in migraine: Results from twins reared apart and twins reared together. Headache J Headache Pain 43(3):235–244

Svensson DA, Waldenlind E, Ekbom K, Pedersen NL (2004) Heritability of migraine as a function of definition. Headache J Headache Pain 5(3):171

Vanmolkot KR, Kors EE, Hottenga JJ, Terwindt GM, Haan J, Hoefnagels WA, Black DF, Sandkuijl LA, Frants RR, Ferrari MD (2003) Novel mutations in the na+, k+-atpase pump gene atp1a2 associated with familial hemiplegic migraine and benign familial infantile convulsions. Ann Neurol 54(3):360–6

Volk HE, Neuman RJ, Todd RD (2005) A systematic evaluation of adhd and comorbid psychopathology in a population-based twin sample. J Am Acad Child Psy 44(8):768(8)

Wessman M, Kallela M, Kunisto MA, Marttila P, Sobel E, Hartiala J, Oswell G, Leal SM, Papp JC, Hämäläinen E, Broas P, Joslyn G, Hovatta I, Hiekkalinna T, Kaprio J, Ott J, Cantor RM, Zwart JA, Ilmavirta M (2002) A susceptibility locus for migraine with aura, on chromosome 4q24. Am J Hum Genet 70(3):652

Wessman M, Terwindt GM, Kaunisto MA, Palotie A, Ophoff RA (2007) Migraine: a complex genetic disorder. Lancet Neurol 6(6):521–532

Whittemore AS, Halpern J (1994) A class of tests for linkage using affected pedigree members. Biometrics 50(1):118–127

Woodbury MA, Clive J, Garson A Jr (1978) Mathematical typology: a grade of membership technique for obtaining disease definition. Comput Biomed Res 11(3):277–98

Zhu G, Evans DM, Duffy DL, Montgomery GW, Medland SE, Gillespie NA, Ewen KR, Jewell M, Liew YW, Hayward NK (2004) A genome scan for eye color in 502 twin families: most variation is due to a qtl on chromosome 15q. Twin Res 7(2):197–210

Ziegler DK, Hur Y-M, Bouchard TJ, Hassanein RS, Barter R (1998) Migraine in twins raised together and apart. Headache J Head Face Pain 38(6):417–422