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The Genetics and Comorbidity of Migraine

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I

Migraine epidemiology and pathophysiology

Introduction

What constitutes migraine, is not a trivial question. The characteristics of migraine vary greatly, both between patients and between attacks. A diagnosis of migraine used to be based on clinical experts' opinions. No precise criteria existed as to which number and combination of symptoms were required for a diagnosis. This made it difficult to compare one case to another. For this reason, it was decided that operational diagnostic criteria were necessary to improve and advance headache research. This was achieved in 1988 with the publication of the first edition of the International Classification of Headache Disorders (ICHD) by the International Headache Society (IHS; Headache Classification Committee of the International Headache Society, 1988). In 2004, a revised edition (ICHD-II) was published (Headache Classification Committee of the International Headache Society, 2004). The ICHD classification distinguishes primary and secondary headaches: primary headaches are headaches with no cause other than the headache disorder itself, whereas secondary headaches are headaches resulting from another disorder. The most common primary headaches are migraine and tension type headache. Other primary headaches are cluster headache and various other, less prevalent headache subtypes. This thesis will focus entirely on migraine. Migraine has various subtypes, the most common being migraine with aura (MA; ICHD-II 1.2) and migraine without aura (MO; ICHD-II 1.1). This distinction will be discussed in more detail in Chapter 5. Table 1.1 shows the diagnostic criteria for these subtypes, according to the second edition of the IHS classification. About one third of migraineurs have attacks of migraine with aura, but most MA patients have a relatively low aura frequency (Kelman, 2004).

The phases of a migraine attack

Typically, a migraine attack has several phases. The headache phase is often preceded by a prodrome (sometimes called pre-headache or premonitory phase), during which patients experience premonitory ('warning') symptoms, such as changes in mood or behavior (Bigal et al., 2009). Specific symptoms often reported are fatigue, yawning, and phonophobia (Schoonman et al., 2006). Craving certain foods can also be part of the prodrome (Blau, 1992).

In a subgroup of patients, the headache phase is preceded by aura symptoms. Auras can occur in different modalities; by far the most common is the visual aura, which is reported by almost all patients who experience some form of aura, (Eriksen et al., 2004), but sensory and aphasic auras are also

reported. Visual aura symptoms are often described as 'flickering lights', 'zigzag patterns', 'blind spots' (also called scotomas), or other visual disturbances, that slowly move through the visual field. Sensory aura symptoms are commonly characterized as 'tingling sensations in the limbs' or 'numbness'. Some patients experience aphasic speech disturbances (e.g. having trouble finding the right words). While visual aura symptoms frequently occur alone, sensory and aphasic aura usually occur in combination with at least one other type of aura. Usually, the different types of aura occur in succession (Eriksen et al., 2004), with the visual aura occurring first, followed by the sensory aura, and then by the aphasic aura (Cutrer & Huerter, 2007).

During or after the aura, the headache phase sets in. This phase can last several hours to several days. Migraine headaches are typically characterized by pounding, pulsating headache, which is often unilateral, moderate or severe in pain intensity and aggravated by routine physical activities such as walking stairs. Migraine headaches are commonly accompanied by nausea and/or vomiting, photophobia (hypersensitivity to light) and phonophobia (hypersensitivity to sound). The headache phase is followed by a resolution phase and often a postdromal 'hangover' (Blau, 1992).

TABLE 1.1

The diagnostic criteria for migraine with and without aura, as defined by the International Headache Society (2004)	
Migraine without aura (1.1)	
A.	At least 5 attacks fulfilling criteria B-D
B.	Headache attacks lasting 4-72 hours (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

Table 1.1 (continued)

Migraine with aura (1.2)	
A.	At least 2 attacks fulfilling criterion B
B.	Migraine aura fulfilling criteria B and C for one of the subforms 1.2.1-1.2.6*
C.	Not attributed to another disorder

Typical aura with migraine headache (1.2.1)	
A.	At least 2 attacks fulfilling criteria B-D
B.	Aura consisting of at least one of the following, but no motor weakness:
	1. fully reversible visual symptoms including positive features (e.g., flickering lights, spots or lines) and/or negative features (i.e., loss of vision)
	2. fully reversible sensory symptoms including positive features (i.e., pins and needles) and/or negative features (i.e., numbness)
C.	At least two of the following:
	1. homonymous visual symptoms and/or unilateral sensory symptoms
	2. at least one aura symptom develops gradually over >= 5 minutes and/or different aura symptoms occur in succession over >= 5 minutes
	3. each symptom lasts >= 5 and <= 60 minutes
D.	Headache fulfilling criteria B-D for 1.1 Migraine without aura begins during the aura or follows aura within 60 minutes
E.	Not attributed to another disorder

* 1.2.1 Typical aura with migraine headache; 1.2.2 Typical aura with non-migraine headache; 1.2.3 Typical aura without headache; 1.2.4 Familial hemiplegic migraine (FHM); 1.2.5 Sporadic hemiplegic migraine; 1.2.6 Basilar-type migraine. 1.2.1 is the subtype referred to as 'migraine with aura' throughout this thesis.

Impact and costs to society

Migraine is a disabling condition with a big impact on patients' lives. During a migraine attack, patients are usually unable to continue their normal activities and will typically stay in bed for the duration of the attack. Therefore, especially when the attack frequency is high, the disorder can badly interfere with the patient's work and social life.

Migraine headaches are undertreated and underdiagnosed; many patients never consult a physician, although estimates differ across studies and populations. For instance, in a French study (Lantéri-Minet et al., 2005), it was

reported that around 40% of migraineurs had never consulted a physician for their migraine, whereas a study in a US population reported almost 80% for headaches in general (Linet et al., 1989).

Due to the fact that many patients do not seek medical attention, the direct costs of migraine are comparatively low, but still quite substantial due to the high prevalence of the disorder. The indirect costs due to lost or reduced productivity are enormous. In a 2004 study the total annual costs of migraine were estimated at around 2 billion euros per year in the Netherlands only, and around 27 billion euros for all European countries together; 1.5 billion due to direct (healthcare) costs and 25.5 billion due to indirect costs (Andlin-Sobocki et al., 2005).

Prevalence and risk factors

One of the reasons for the high costs of migraine is its high prevalence. In a large US population-based study (N = 20,468) a 1-year prevalence of 18% in females and 6% in males was observed (Stewart et al., 1992). A study in a Dutch population sample (N = 6491) reported a lifetime prevalence of 33% in women and 13.3% in men. The 1-year prevalence was 25% and 7.5%, respectively (Launer et al., 1999). These are only the individuals who meet full IHS criteria for migraine with or without aura. Including individuals with probable migraine (i.e. migraine fulfilling all but one of the A-D criteria) results in a prevalence almost twice as high. A French study reported a prevalence of strict migraine of 16% in females and 6% in males; another 12% and 8% fulfilled criteria for probable migraine, respectively (Lantéri-Minet et al., 2005).

The onset of the disorder is usually in early adolescence, with a peak incidence between 10 and 15 years of age (Stewart et al., 1991). The prevalence is highest between 30 and 40 and then gradually decreases (Stewart et al., 1992). This pattern is most distinctive in females, with high prevalences between adolescence and menopause. In addition, many women have migraine attacks related to the menstrual cycle, which is referred to as menstrual migraine (Lay & Broner, 2009). Together with the 2-3 times higher prevalence of migraine in women, these observations strongly suggest a key role for hormones in the pathogenesis of migraine. Migraine attacks appear to be triggered particularly by sudden drops or increases in estrogen levels, which may explain the often reported decrease in migraine frequency during pregnancy, followed by a return of the migraine after delivery (Brandes, 2006).

The role of precipitating factors in causing migraine attacks remains somewhat controversial. In a study of migraine precipitants in clinical migraine

patients in the US, about three-quarters of all migraineurs reported that their migraines were at least occasionally caused by triggers. The most reported precipitating factors in this study were stress, hormones, not eating, weather and sleep disturbances (Kelman, 2007). Although patient reports of triggers are numerous, it is often unclear whether reported triggers are indeed causally involved. For instance, Schoonman et al. (2007) investigated the relationship between migraine and stress. They studied both perceived stress and objectively measured biological stress reactions. Although patients reported an increase in perceived stress in the days before an attack, they found no evidence for a biological stress response before or during migraine. This might be due to study limitations; however, one alternative explanation the authors suggest is that prior to the attack patients may perceive situations as more stressful, as a consequence of prodromal brain changes. The latter might also be the case for some other reported triggers. For instance, craving for sweet food is known to be a prodromal symptom; thus eating chocolate may be a symptom rather than a cause (Blau, 1992). Clearly however, this does not exclude the possibility that some reported trigger factors are truly causally involved in migraine attacks.

Pathophysiology

It used to be thought that migraine was primarily a vascular disorder. Vascular changes were viewed as the primary cause of migraine attacks. However, this view became untenable when experiments showed that vasodilation can be induced without necessarily causing a migraine attack (Kruuse et al., 2003), while other studies showed that migraine can be induced without being accompanied by any vascular changes (e.g. Schoonman et al., 2008). These findings indicate that vascular changes are neither necessary nor sufficient for a migraine attack to occur, suggesting they are merely an epiphenomenon (Goadsby, 2009).

These days the dominating view is that migraine is a typical neurological condition. Although it remains speculative how exactly they relate to each other, three processes are important in migraine: 1) cortical spreading depression, 2) the activation of the trigeminovascular system (TGVS) and 3) the sensitisaton of peripheral and central brain areas.

Cortical spreading depression and the migraine aura

Through the years, evidence has accumulated that the migraine aura is most likely caused by a brain event called cortical spreading depression (CSD). This phenomenon was first observed in a rabbit (Leao, 1944), and can also be

triggered in other laboratory animals. During CSD, a wave of intense depolarization, starting in the occipital lobe, propagates through the brain at a speed of approximately 2-5 mm/min., and is followed by a period of suppressed activity. This corresponds well with the progression of aura symptoms and explains both the positive (scintillations, tingling sensations) and the negative symptoms (scotomas and numbness) of the migraine aura. It may also explain why the different types of aura occur in succession, starting with a visual aura; this is consistent with the progression of the CSD wave through the different cortical areas, starting in the occipital lobe (Lauritzen, 2001).

Further evidence for the involvement of CSD in the migraine aura comes from animal studies. Hadjikhani et al. (2001) showed that CSD in experimental animals is associated with certain changes in cerebral blood flow (CBF), which are very similar to those observed in humans during a migraine aura.

THE TRIGEMINOVASCULAR SYSTEM

Within the skull, pain sensitivity is primarily restricted to the meningeal blood vessels (Pietrobon & Striessnig, 2003), and this is where the headache in migraine is thought to originate. Most likely, the headache phase starts with some neural disturbance that activates the trigeminovascular system [TGVS] (Goadsby et al., 2002). The TGVS consists of the meningeal vessels, which are innervated by the first (opthalmic) division of the trigeminal nerve. The trigeminal nerve projects to nuclei in the brain stem, such as the trigeminal nucleus caudalis (TNC), which in turn project to higher brain centers, including thalamus, hypothalamus and cortex. Activation of the TGVS causes the release of neuropeptides (including calcitonin gene-related peptide [CGRP] and substance P) from the peripheral trigeminal nerve endings (Goadsby et al., 1988). These neuropeptides are thought to play a role in causing and maintaining the headache (Bigal et al., 2009). The trigeminal afferents carry the pain signal via the brain stem to higher brain centers involved in the perception of pain (Pietrobon & Striessnig, 2003).

PERIPHERAL AND CENTRAL SENSITIZATION

It is thought that the throbbing, pulsating nature of migraine headache, and the aggravation of the headache by activities that increase cranial pressure (e.g. walking stairs or coughing), are caused by a process of peripheral sensitization (Silberstein, 2004). Another important symptom often observed in migraine patients is cutaneous allodynia [i.e. a sensation of pain caused by stimuli that are not normally painful (see Burstein et al., 2000)]. This is thought to result

from central sensitization of neurons in the TNC, which receive input from dura and skin (Silberstein, 2004). Central sensitization is thought to play an important role in the later stages of the migraine attack (Bigal et al., 2009).

The connection between aura and headache

The view that the aura is caused by CSD has become generally accepted, and the same is true for the view that activation of the TGVS underlies migraine headache. However, the relationship between the aura and the activation of the TGVS and the start of the headache remains elusive. It has been hypothesized that CSD might activate the TGVS, and in a study with rats it was demonstrated that this is indeed possible (Bolay et al., 2002). The aura might thus be the cause of the migraine attack. This theory has been criticized, however, because it does not explain what happens in the majority of migraineurs who do not experience aura symptoms (Goadsby, 2001). It has been suggested that in these patients a 'silent aura' might occur, but there is limited evidence supporting this theory (see Sanchez-Del-Rio et al., 2006). Others argue that aura cannot be the trigger of migraine headache, due to the existence of aura without headache (ICHD-II 1.2.3; typical aura without headache) in some patients (Goadsby, 2009). Interestingly, however, Hauge et al. (2009), who recently investigated the effect of the newly developed CSD-inhibiting drug tonabersat reported that this drug prevents attacks with, but not attacks without aura in MA patients. If tonabersat indeed prevents migraine headache by preventing the aura, this would strongly point towards a causal role of aura in the subset of migraine attacks that are preceded by aura symptoms. However, the results of this relatively small study require replication at a larger scale to assess the potential implications of these findings.

Comorbidity

A fascinating feature of migraine is its higher than expected co-occurrence (comorbidity) with many other disorders. A wide range of conditions have been reported to be comorbid with migraine, including psychiatric disorders such as bipolar disorder, panic disorder and phobias, but also non-psychiatric conditions such as other chronic pain conditions, epilepsy, stroke, certain congenital heart defects and endometriosis (e.g. P. Anttila et al., 2001; Breslau et al., 1991; Hagen et al., 2002; Lamy et al., 2002; Merikangas et al., 1990; Merikangas et al., 1997; Nyholt et al., 2009; Von Korff et al., 2005). The reasons for these comorbidities remain largely unknown. The disorders could be

causally related, or share genetic and/or environmental risk factors, but in general, little is known about the mechanisms of comorbidity.
The disorder most studied in the context of migraine comorbidity is undoubtedly depression. It has been suggested that for instance the serotonin or dopamine systems might be involved in both migraine and depression and hence explain the association between them (Breslau et al., 1991; Frediani & Villani, 2007). In a longitudinal study it was found that migraine and depression were bidirectionally related; migraineurs had an increased risk of developing depression, and vice versa (Breslau et al., 2000). Furthermore, it was recently shown in a bivariate genetic study of migraine and depression that the two traits were in part explained by shared genetic and non-shared environmental risk factors (Schur et al., 2009). The comorbidity of migraine and depression is investigated in more detail in Chapters 6 and 7 of this thesis.

Genetics

The heritability of migraine is undisputed. It was observed long ago that the disorder runs in families, and early twin studies noticed a higher concordance rate for migraine in monozygotic than in dizygotic twins, pointing towards a heritable component (Russell & Olesen, 1993). In a large analysis of twins in six European countries, including a total of 29,717 participants it was estimated that the heritability of migraine is approximately 40-50%. There was some indication that non-additive genetic effects may play a role but most individual studies lacked the power to detect this. Shared environmental factors do not seem to be important in migraine (Mulder et al., 2003). The common migraines (i.e. MO and MA) are most likely polygenic disorders. They do not show a distinctive pattern of inheritance such as observed in a classical Mendelian trait, influenced by a single gene. This is most likely the reason it has proven very difficult to find causative genes for common migraine.

Familial Hemiplegic migraine

Unlike common migraine, Familial Hemiplegic Migraine (FHM) follows a Mendelian pattern of inheritance. This is the reason why most of our knowledge of migraine genetics comes from FHM studies. FHM is a rare and severe type of MA, characterized by temporary hemiplegia (i.e. motor weakness or loss of motor function on one side of the body) during the aura phase. Several mutations in 3 different genes have been identified that can cause FHM.

The first FHM gene was originally mapped to chromosome 19p13 in a parametric linkage study on two large FHM pedigrees (Joutel et al., 1993).

Several years later it was determined that the causative gene was the calcium channel, voltage-dependent, P/Q type, alpha 1A subunit (*CACNA1A*; Ophoff et al., 1996). Mutations in this gene are responsible for the disorder in a substantial percentage of FHM families, although estimates differ between studies (Joutel et al., 1994; Ophoff et al., 1994; Thomsen et al., 2007). The second FHM gene is the ATPase, Na+/K+ transporting, alpha 2 polypeptide *(ATP1A2)*. This gene was identified in 2003 by De Fusco et al., after several linkage studies had already reported linkage to chromosome 1q23 (Ducros et al., 1997; Marconi et al., 2003). The *ATP1A2* gene codes for the alpha-2 subunit of a NA+/K+ pump. Finally, in a genome-wide linkage analysis, Dichgans et al. (2005) mapped a third FHM locus to chromosome 2q24. They determined that the causal mutation was located in the sodium channel, voltage-gated, type I, alpha subunit *(SCN1A)* on chromosome 2q24. This gene codes for the alpha-1 subunit of a voltage-gated sodium channel. Interestingly, *SCN1A* has previously been implicated in epilepsy, which is fascinating given the reported comorbidity between migraine and epilepsy.

A possible mechanism by which these mutations affect FHM is by increasing the glutamate and potassium levels in the synaptic cleft, thus facilitating CSD (van den Maagdenberg et al., 2007). What the FHM mutations have in common is that they are all related to the functioning of ion channels. This has led to the hypothesis that not only FHM, but perhaps also common migraine could be viewed as a channelopathy. Whether ion channels play an important role in common migraine is still under investigation, however a recent association study of 155 ion channel genes did not find convincing evidence for their involvement in MO or MA (Nyholt et al., 2008).

Common migraine: gene-finding studies

The search for genes underlying common migraine has not been as successful as for FHM. As mentioned earlier, this is most likely due to the fact that common migraine is a complex disorder, influenced by many genes of small effect. In addition, the disorder may be genetically heterogeneous, i.e. different genes may underlie the phenotype in different individuals. Gene-finding studies for migraine commonly apply several different methods such as linkage analysis and candidate-gene association studies. These methods are described in more detail in Chapter 2.

Linkage

Through the years, many linkage studies of migraine have been conducted in an attempt to localize genes for common migraine. Unfortunately, many results to date have remained unreplicated. However, with the increasing number of studies, several consistently replicated loci are beginning to emerge, for instance on chromosome 4q24 (V. Anttila et al., 2006; Wessman et al., 2002) and chromosome 10q22 (V. Anttila et al., 2006; V. Anttila et al., 2008; Nyholt et al., 2005). Thus, evidence is increasing that these regions indeed harbour one or more genes involved in common migraine. For a recent overview of migraine linkage results, see Oedegaard et al. (2009). A disadvantage of linkage studies is that they can often only provide a global indication of where a causative gene might be located. To date, the actual genes causing the reported linkage signals remain unidentified.

Candidate gene association studies

Association studies of migraine generally focus on several types of candidate genes. Genes coding for ion channels are of interest due to the findings in FHM, which all implicate ion channels. Genes involved in vascular function are studied because of the vascular changes observed in migraine and the association of migraine and stroke. Genes related to hormone function (e.g. estrogen and progesterone receptors) are candidates because of the sex differences in migraine prevalence and the observed changes related to 'hormonal milestones', and genes involved in neurotransmitter function (e.g. dopamine and serotonin receptors), could be candidates due to the suspected involvement of neurotransmitter pathways in migraine pathophysiology. One of the more consistent findings is the association of migraine (particularly migraine with aura) with the 5,10-methylenetetrahydrofolate reductase (NADPH) *[MTHFR]* gene (e.g. Kowa et al., 2000; Rubino et al., 2009; Scher et al., 2006). However, in general, many initially positive association results for migraine failed to replicate consistently in follow-up studies (for a review, see Colson et al., 2007; de Vries et al., 2009). A likely explanation for the lack of success of the candidate gene method in migraine research is that our knowledge of migraine etiology is too limited to allow the identification of good candidates and/or the use of small samples with insufficient power.

Genome-wide association

A recent promising development in genetics research has been the introduction of genome-wide association (GWA) studies, the first of which started to be published in 2005. Since then, hundreds have followed and many disease-associated genes have been identified (Manolio & Collins, 2009). Due to the increased resolution compared to linkage studies, and the fact that prior hypotheses on the genes involved are not necessary, GWA studies hold some promise to detect common variants associated with migraine. However, to detect loci with a small effect, very large samples are needed (Visscher, 2008). This has recently led to the formation of large GWA consortia, to bring together the sample sizes necessary to identify significant disease associations.

Overview of the chapters in this thesis

In this thesis, I will first provide a short overview of the genetic epidemiological methods used in the different chapters (Chapter 2). Chapter 3 summarizes the ascertainment procedures for the data this thesis is based on, including details on the headache questionnaire that was used to assess migraine status. In Chapter 4, it is investigated whether and how a potential non-response bias might influence the collected data.

In the second section, the migraine phenotype and its relationship with depression is explored in more detail. Chapter 5 describes how the questionnaire data were analysed with latent class analysis (LCA) to investigate whether subtypes of migraine could be identified, and how the resulting classification was used as the phenotype in an analysis of the genetic architecture of migraine. In Chapter 6 migraine characteristics are compared between depressed and non-depressed individuals, while Chapter 7 explores whether migraine and anxious depression are genetically correlated, and how this association might be explained.

The third section addresses the issue of gene finding for migraine. A linkage study and a meta-analysis of genome-wide association studies are described (Chapters 8 and 9, respectively). Finally, in Chapter 10, an attempt is made to summarize the results of these studies into a coherent conclusion about the outcomes of this thesis and suggest future directions of research aimed at identifying genetic factors underlying an individual's susceptibility to common migraine.

References

Andlin-Sobocki, P., Jonsson, B., Wittchen, H. U., & Olesen, J. (2005). Cost of disorders of the brain in Europe. Eur J Neurol, 12 Suppl 1, 1-27.

Anttila, P., Metsahonkala, L., Mikkelsson, M., Helenius, H., & Sillanpaa, M. (2001). Comorbidity of other pains in schoolchildren with migraine or nonmigrainous headache. J Pediatr, 138(2), 176-180.

Anttila, V., Kallela, M., Oswell, G., Kaunisto, M. A., Nyholt, D. R., Hamalainen, E., et al. (2006). Trait components provide tools to dissect the genetic susceptibility of migraine. Am J Hum Genet, 79(1), 85-99.

Anttila, V., Nyholt, D. R., Kallela, M., Artto, V., Vepsalainen, S., Jakkula, E., et al. (2008). Consistently replicating locus linked to migraine on 10q22-q23. Am J Hum Genet, 82(5), 1051-1063.

Bigal, M. E., Ferrari, M., Silberstein, S. D., Lipton, R. B., & Goadsby, P. J. (2009). Migraine in the triptan era: lessons from epidemiology, pathophysiology, and clinical science. Headache, 49 Suppl 1, S21-33.

Blau, J. N. (1992). Migraine: theories of pathogenesis. Lancet, 339(8803), 1202.

Bolay, H., Reuter, U., Dunn, A. K., Huang, Z., Boas, D. A., & Moskowitz, M. A. (2002). Intrinsic brain activity triggers trigeminal meningeal afferents in a migraine model. Nat Med, 8(2), 136-142.

Brandes, J. L. (2006). The influence of estrogen on migraine: a systematic review. Jama, 295(15), 1824-1830.

Breslau, N., Davis, G. C., & Andreski, P. (1991). Migraine, psychiatric disorders, and suicide attempts: an epidemiologic study of young adults. Psychiatry Res, 37(1), 11-23.

Breslau, N., Schultz, L. R., Stewart, W. F., Lipton, R. B., Lucia, V. C., & Welch, K. M. (2000). Headache and major depression: is the association specific to migraine? Neurology, 54(2), 308-313.

Burstein, R., Yarnitsky, D., Goor-Aryeh, I., Ransil, B. J., & Bajwa, Z. H. (2000). An association between migraine and cutaneous allodynia. Ann Neurol, 47(5), 614-624.

Colson, N. J., Fernandez, F., Lea, R. A., & Griffiths, L. R. (2007). The search for migraine genes: an overview of current knowledge. Cell Mol Life Sci, 64(3), 331-344.

Cutrer, F. M., & Huerter, K. (2007). Migraine aura. Neurologist, 13(3), 118-125.

De Fusco, M., Marconi, R., Silvestri, L., Atorino, L., Rampoldi, L., Morgante, L., et al. (2003). Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. Nat Genet, 33(2), 192-196.De Vries, B., Frants, R. R., Ferrari, M. D., & van den Maagdenberg, A. M. (2009). Molecular genetics of migraine. Hum Genet, 126(1), 115-132.

Dichgans, M., Freilinger, T., Eckstein, G., Babini, E., Lorenz-Depiereux, B., Biskup, S., et al. (2005). Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. Lancet, 366(9483), 371-377.

Ducros, A., Joutel, A., Vahedi, K., Cecillon, M., Ferreira, A., Bernard, E., et al. (1997). Mapping of a second locus for familial hemiplegic migraine to 1q21-q23 and evidence of further heterogeneity. Ann Neurol, 42(6), 885-890.

Eriksen, M. K., Thomsen, L. L., Andersen, I., Nazim, F., & Olesen, J. (2004). Clinical characteristics of 362 patients with familial migraine with aura. Cephalalgia, 24(7), 564-575.

Frediani, F., & Villani, V. (2007). Migraine and depression. Neurol Sci, 28 (Suppl 2), S161-165.

Goadsby, P. J. (2001). Migraine, aura, and cortical spreading depresssion: why are we still talking about it? Annals of Neurology, 49(2), 4.

Goadsby, P. J. (2009). Pathophysiology of migraine. Neurol Clin, 27(2), 335-360.

Goadsby, P. J., Edvinsson, L., & Ekman, R. (1988). Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system. Ann Neurol, 23(2), 193-196.

Goadsby, P. J., Lipton, R. B., & Ferrari, M. D. (2002). Migraine--current understanding and treatment. N.Engl.J Med, 346(4), 257.

Hadjikhani, N., Sanchez Del Rio, M., Wu, O., Schwartz, D., Bakker, D., Fischl, B., et al. (2001). Mechanisms of migraine aura revealed by functional MRI in human visual cortex. Proc Natl Acad Sci U S A, 98(8), 4687-4692.

Hagen, K., Einarsen, C., Zwart, J. A., Svebak, S., & Bovim, G. (2002). The co-occurrence of headache and musculoskeletal symptoms amongst 51 050 adults in Norway. Eur J Neurol, 9(5), 527-533.

Hauge, A. W., Asghar, M. S., Schytz, H. W., Christensen, K., & Olesen, J. (2009). Effects of tonabersat on migraine with aura: a randomised, double-blind, placebo-controlled crossover study. Lancet Neurol, 8(8), 718-723.

Headache Classification Committee of the International Headache Society (1988). Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain, Cephalalgia (Vol. 8 [Suppl 7], pp. 1-96).

Headache Classification Committee of the International Headache Society (2004). The International Classification of Headache Disorders: 2nd edition, Cephalalgia (Vol. 24 [Suppl 1], pp. 9-160).

Joutel, A., Bousser, M. G., Biousse, V., Labauge, P., Chabriat, H., Nibbio, A., et al. (1993). A gene for familial hemiplegic migraine maps to chromosome 19. Nat Genet, 5(1), 40-45.

Joutel, A., Ducros, A., Vahedi, K., Labauge, P., Delrieu, O., Pinsard, N., et al. (1994). Genetic heterogeneity of familial hemiplegic migraine. Am J Hum Genet, 55(6), 1166-1172.

Kelman, L. (2004). The aura: a tertiary care study of 952 migraine patients. Cephalalgia, 24(9), 728-734.

Kelman, L. (2007). The triggers or precipitants of the acute migraine attack. Cephalalgia, 27(5), 394-402.

Kowa, H., Yasui, K., Takeshima, T., Urakami, K., Sakai, F., & Nakashima, K. (2000). The homozygous C677T mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for migraine. Am J Med Genet, 96(6), 762-764.

Kruuse, C., Thomsen, L. L., Birk, S., & Olesen, J. (2003). Migraine can be induced by sildenafil without changes in middle cerebral artery diameter. Brain, 126(Pt 1), 241-247.

Lamy, C., Giannesini, C., Zuber, M., Arquizan, C., Meder, J. F., Trystram, D., et al. (2002). Clinical and imaging findings in cryptogenic stroke patients with and without patent foramen ovale: the PFO-ASA Study. Atrial Septal Aneurysm. Stroke, 33(3), 706-711.

Lantéri-Minet, M., Valade, D., Geraud, G., Chautard, M. H., & Lucas, C. (2005). Migraine and probable migraine--results of FRAMIG 3, a French nationwide survey carried out according to the 2004 IHS classification. Cephalalgia, 25(12), 1146-1158.

Launer, L. J., Terwindt, G. M., & Ferrari, M. D. (1999). The prevalence and characteristics of migraine in a population-based cohort: the GEM study. Neurology, 53(3), 537-542.

Lauritzen, M. (2001). Cortical spreading depression in migraine. Cephalalgia, 21(7), 757.

Lay, C. L., & Broner, S. W. (2009). Migraine in women. Neurol Clin, 27(2), 503-511.

Leao, A. A. P. (1944). Spreading depression of activity in the cerebral cortex. J Neurophysiol, 7, 359.

Linet, M. S., Stewart, W. F., Celentano, D. D., Ziegler, D., & Sprecher, M. (1989). An epidemiologic study of headache among adolescents and young adults. Jama, 261(15), 2211-2216.

Manolio, T. A., & Collins, F. S. (2009). The HapMap and genome-wide association studies in diagnosis and therapy. Annu Rev Med, 60, 443-456.

Marconi, R., De Fusco, M., Aridon, P., Plewnia, K., Rossi, M., Carapelli, S., et al. (2003). Familial hemiplegic migraine type 2 is linked to 0.9Mb region on chromosome 1q23. Ann Neurol, 53(3), 376-381.

Merikangas, K. R., Angst, J., & Isler, H. (1990). Migraine and psychopathology. Results of the Zurich cohort study of young adults. Arch Gen Psychiatry, 47(9), 849-853.

Merikangas, K. R., Fenton, B. T., Cheng, S. H., Stolar, M. J., & Risch, N. (1997). Association between migraine and stroke in a large-scale epidemiological study of the United States. Arch Neurol, 54(4), 362-368.

Mulder, E. J., Van Baal, C., Gaist, D., Kallela, M., Kaprio, J., Svensson, D. A., et al. (2003). Genetic and environmental influences on migraine: a twin study across six countries. Twin Res, 6(5), 422-431.

Nyholt, D. R., Gillespie, N. G., Merikangas, K. R., Treloar, S. A., Martin, N. G., & Montgomery, G. W. (2009). Common genetic influences underlie comorbidity of migraine and endometriosis. Genet Epidemiol, 33(2), 105-113.

Nyholt, D. R., LaForge, K. S., Kallela, M., Alakurtti, K., Anttila, V., Farkkila, M., et al. (2008). A high-density association screen of 155 ion transport genes for involvement with common migraine. Hum Mol Genet, 17(21), 3318-3331.

Nyholt, D. R., Morley, K. I., Ferreira, M. A., Medland, S. E., Boomsma, D. I., Heath, A. C., et al. (2005). Genomewide significant linkage to migrainous headache on chromosome 5q21. Am J Hum Genet, 77(3), 500-512.

Oedegaard, K. J., Greenwood, T. A., Lunde, A., Fasmer, O. B., Akiskal, H. S., & Kelsoe, J. R. (2009). A genome-wide linkage study of bipolar disorder and co-morbid migraine: Replication of migraine linkage on chromosome 4q24, and suggestion of an overlapping susceptibility region for both disorders on chromosome 20p11. J Affect Disord.

Ophoff, R. A., Terwindt, G. M., Vergouwe, M. N., van Eijk, R., Oefner, P. J., Hoffman, S. M., et al. (1996). Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell, 87(3), 543-552.

Ophoff, R. A., van Eijk, R., Sandkuijl, L. A., Terwindt, G. M., Grubben, C. P., Haan, J., et al. (1994). Genetic heterogeneity of familial hemiplegic migraine. Genomics, 22(1), 21-26.

Pietrobon, D., & Striessnig, J. (2003). Neurobiology of Migraine. Nature-Reviews-Neuroscience, 4(5), 386.

Rubino, E., Ferrero, M., Rainero, I., Binello, E., Vaula, G., & Pinessi, L. (2009). Association of the C677T polymorphism in the MTHFR gene with migraine: a meta-analysis. Cephalalgia, 29(8), 818-825.

Russell, M. B., & Olesen, J. (1993). The genetics of migraine without aura and migraine with aura. Cephalalgia, 13(4), 245-248.

Sanchez-Del-Rio, M., Reuter, U., & Moskowitz, M. A. (2006). New insights into migraine pathophysiology. Curr Opin Neurol, 19(3), 294-298.

Scher, A. I., Terwindt, G. M., Verschuren, W. M., Kruit, M. C., Blom, H. J., Kowa, H., et al. (2006). Migraine and MTHFR C677T genotype in a population-based sample. Ann Neurol, 59(2), 372-375.

Schoonman, G. G., Evers, D. J., Ballieux, B. E., de Geus, E. J., de Kloet, E. R., Terwindt, G. M., et al. (2007). Is stress a trigger factor for migraine? Psychoneuroendocrinology, 32(5), 532-538.

Schoonman, G. G., Evers, D. J., Terwindt, G. M., van Dijk, J. G., & Ferrari, M. D. (2006). The prevalence of premonitory symptoms in migraine: a questionnaire study in 461 patients. Cephalalgia, 26(10), 1209-1213.

Schoonman, G. G., van der Grond, J., Kortmann, C., van der Geest, R. J., Terwindt, G. M., & Ferrari, M. D. (2008). Migraine headache is not associated with cerebral or meningeal vasodilatation--a 3T magnetic resonance angiography study. Brain, 131(Pt 8), 2192-2200.

Schur, E. A., Noonan, C., Buchwald, D., Goldberg, J., & Afari, N. (2009). A Twin Study of Depression and Migraine: Evidence for a Shared Genetic Vulnerability. Headache, 49(10), 1493-1502.

Silberstein, S. D. (2004). Migraine pathophysiology and its clinical implications. Cephalalgia, 24 Suppl 2, 2-7.

Stewart, W. F., Linet, M. S., Celentano, D. D., Van Natta, M., & Ziegler, D. (1991). Age- and sex-specific incidence rates of migraine with and without visual aura. Am J Epidemiol, 134(10), 1111-1120.

Stewart, W. F., Lipton, R. B., Celentano, D. D., & Reed, M. L. (1992). Prevalence of migraine headache in the United States. Relation to age, income, race, and other sociodemographic factors. Jama, 267(1), 64-69.

Thomsen, L. L., Kirchmann, M., Bjornsson, A., Stefansson, H., Jensen, R. M., Fasquel, A. C., et al. (2007). The genetic spectrum of a population-based sample of familial hemiplegic migraine. Brain, 130(Pt 2), 346-356.

Van den Maagdenberg, A. M., Haan, J., Terwindt, G. M., & Ferrari, M. D. (2007). Migraine: gene mutations and functional consequences. Curr Opin Neurol, 20(3), 299-305.

Visscher, P. M. (2008). Sizing up human height variation. Nat Genet, 40(5), 489-490.

Von Korff, M., Crane, P., Lane, M., Miglioretti, D. L., Simon, G., Saunders, K., et al. (2005). Chronic spinal pain and physical-mental comorbidity in the United States: results from the national comorbidity survey replication. Pain, 113(3), 331-339.

Wessman, M., Kallela, M., Kaunisto, M. A., Marttila, P., Sobel, E., Hartiala, J., et al. (2002). A susceptibility locus for migraine with aura, on chromosome 4q24. Am J Hum Genet, 70(3), 652-662.

2

Genetic Epidemiology - How to quantify, localize and identify genetic influences on human traits

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Introduction

Individual differences in psychological or behavioral traits can be explained by a combination of genetic and environmental differences between individuals. When a trait is said to be highly genetic, this means that a large proportion of the variance in the trait is explained by genetic factors, i.e. the effect of one or many genes that each have their influence on the expression of the trait. Environmental factors can range from intrauterine environment to the influence of the family environment, school, friends and many other unidentified non-genetic factors. In this chapter we will provide an overview of methods used to model the contribution of genes and environment to variance in a trait or a set of traits, and to localize and identify the regions of the genome that may be involved. The area of research that focuses on quantifying genetic effects is called behavior genetics or genetic epidemiology. Genes can be localized and identified with genetic linkage and association methods. Finally, we will discuss factors that influence the expression of genes (such as epigenetic modification) and methods to study how gene expression is regulated and how genes interact.

The reader is assumed to have some basic knowledge of genetic terminology. Genetic information is encoded in DNA (deoxiribonucleic acid) molecules. The DNA code contains the units of genetic information we call genes. There is no real agreement on what exactly defines a gene, the definition has evolved along with the advances in science. Commonly used definitions of a gene are: 'a unit of inheritance' or 'a packet of genetic information that encodes a protein or RNA'. The estimated number of genes in the human genome is also a subject of debate. Not too long ago it was predicted that the human genome contained around 100,000-150,000 genes (e.g., Liang et al., 2000). However, more recent estimates have gone down to 20,000-25,000 (International Human Genome Sequencing Consortium, 2004).

In humans, the DNA molecules are organised in 2 x 23 chromosomes: 22 pairs of autosomes and one pair of sex chromosomes. The genetic sequence as a whole is called the genome, and a location in the genome that for instance contains a gene or a genetic marker is referred to as a locus. A quantitative trait locus (QTL) is a locus that harbours a gene influencing a quantitative trait, i.e. a trait that varies on a quantitative scale. Assessment of the trait may be on an interval or ordinal scale, in which case an underlying quantitative liability is assumed.

The nuclei of nearly all human cells contain two versions of each chromosome, and therefore of each gene. The two corresponding chromo-

somes are called homologous chromosomes. One is received from the mother, the other from the father. In addition, a small amount of DNA is contained in the (maternally inherited) mitochondria. Although most of the human DNA sequence is identical in all individuals, at some loci different versions of the sequence occur. These variants are called alleles. The word allele can refer to a gene variant, but also to versions of a genetic marker or any other fragment of DNA sequence. Individuals who carry the same allele at both homologous chromosomes are called homozygous. Individuals with two different alleles are heterozygous. The two alleles together, either at one or at multiple loci, make up a person's genotype. The term haplotype is used to indicate a combination of alleles at multiple loci that an individual receives from one parent (Ott, 1999). It usually refers to a combination of alleles transmitted close together on the same chromosome. Finally, the observed characteristics of an individual are called phenotypes.

Alleles affecting quantitative traits can exert their effect in various ways. When the alleles act independently, the effects simply add up, in which case we speak of additive genetic effects. When the effect of one allele depends on the effect of another, i.e. there is an interaction between them, they are referred to as non-additive effects. There are several forms of non-additivity. Interactions between two alleles at the same locus are referred to as dominance. When the interaction is between alleles at two different loci, it is referred to as epistasis. An excellent online tutorial by Shaun Purcell that addresses additivity and dominance can be found on http://pngu.mgh.harvard.edu/~purcell/bgim/-index2.html#sgene.

In this chapter we will provide an overview of genetic epidemiological methods and developments, in three sections. The first part will describe the estimation of heritability, as well as some more advanced modeling based on twin methodology. In part II, methodology used to localize and identify genes will be discussed. Finally, in part III, we will focus on the gene expression and epigenetic modification of the DNA.

Part I: Estimating Heritability

It is often observed that human traits run in families. This is not only the case for diseases or physical appearance but can also apply to personality and behavior. The mere fact that a trait is familial, however, does not tell us whether the trait is heritable, since familial resemblance can also be the result of the influence of a shared family environment.

One method to investigate genetic influences is by studying adopted children and their biological and adoptive parents. Similarities between adopted children and their biological parents reflect genetic influences, whereas similarities between adopted children and their adoptive parents reflect the effects of the family environment. However, there are some disadvantages to adoption studies: adoptions are relatively rare, and adoptive children and parents cannot be assumed to be representative of the general population.

The classical twin model

For this reason many studies use data from twins and their families. In twin studies, the resemblance between monozygotic (MZ) and dizygotic (DZ) twins is compared to estimate the contribution of genes, shared environment and non-shared environment to the variance in a trait. This is based on the fact that MZ twins share 100% of their segregating genes, whereas DZ twins share on average 50%. [Note that this percentage refers to the portion of the genome in which variation occurs, since >99% of the genome is identical between humans; this part is therefore entirely shared in both MZ and DZ twins.] In contrast, both MZ and DZ twins share the home environment. This means that differences between MZ twins must be due to non-shared environmental influences, whereas the extent to which MZ twins are more similar than DZ twins reflects the influence of genetic factors. Using these principles, the variance in a trait can be decomposed as due to additive genetic factors (A), common or shared environment (C) and non-shared environment or measurement error (E). In the absence of dominance or epistasis, the percentage of variance in a trait that is explained by additive genetic factors equals the heritability of the trait, which can be estimated by taking twice the difference between the MZ and DZ twin correlation: $h^2 = 2(rMZ - rDZ)$.

When rMZ > 2rDZ, there is evidence for a contribution of non-additive genetic influences, also referred to as genetic dominance (D), which also includes effects of epistasis. In this case, the percentage of variance explained by

A and D together is referred to as the broad-sense heritability, A alone is called the narrow-sense heritability.

The contribution of A, C, D and E to the trait variance can be estimated based on biometrical genetic theory. Discussing the biometrical model in detail is beyond the scope of this chapter, but it is the basis of a few important principles twin models are based on. For a detailed introduction see Falconer and Mackay (1996).

As explained above, the total phenotypic variance of a trait (P) can be decomposed into components explained by A, C, D and E: $V_P = V_A + V_D + V_C + V_E$. We here assume that there is no interaction or correlation between genetic and environmental factors (the covariance between A and D is zero by definition).

The covariance between MZ twins is expressed as: $cov(MZ) = V_A + V_D + V_C$. Since V_E is by definition non-shared variance, it cannot contribute to covariance of family members. V_C is, by definition, shared, and the genetic variance is also entirely shared because MZ twins are genetically identical. The expectation for the DZ twin covariance is expressed as: $cov(DZ) = ½ V_A + ¼V_D + V_C$. On average ½ of the additive genetic variance is shared between DZ twins (and between non-twin siblings). In order to share non-additive variance, two relatives have to share both alleles of a gene, an event that occurs with a probability of ¼ in DZ twins (or full siblings). Figure 2.1 shows a graphical representation of the model that arises from these principles.

STRUCTURAL EQUATION MODELLING

To estimate the contribution of all genetic and environmental factors to a trait and assess their significance, models can be evaluated and compared using structural equation modelling (SEM). The parameters of a model (which include means, variances and covariances) can be estimated using an optimization approach such as maximum likelihood estimation. The relative goodness-of-fit of different models can be assessed by calculating minus twice the log-likelihood (-2LL) of the data given the model, and comparing these values between models. By dropping or equating parameters, the fit of different models can be compared with a likelihood ratio test. Genetic structural equation modelling usually involves a multiple group design in which data from e.g. MZ and DZ twins are analyzed simultaneously and parameters (a, c, d and e in Figure 2.1) are constrained to be equal across groups to ensure identification of the model. Usually, a fully saturated model that includes estimates for all parameters is tested first. Then the significance of parameters can be tested by constraining them to be zero. For instance, it can be tested whether the C factor

has an effect on the variance of the trait, by fixing the c path coefficient at zero and then comparing the original model to the constrained model. When dropping or equating parameters does not result in a significant deterioration of the model fit, this indicates the more parsimonious model fits the data as well as the more complex model. The best model is the most parsimonious model that still provides a good explanation of the observed data. Significance is determined based on the difference in -2LL between two models, which is asymptotically distributed as χ^2. The degrees of freedom of the test are equal to the difference in the number of parameters. For very large samples alternative fit indices have been proposed, such as the RMSEA (Browne & Cudeck, 1993), the Bayesian Information Criterion (BIC; Schwarz, 1978) and the Akaike Information Criterion (AIC; Akaike, 1987).

Twin models and categorical data

In the case of a continuous variable, the trait is assumed to be normally distributed [which is indeed expected for traits that are affected by many genes (Fischer, 1918)]. Clearly, non-continuous phenotypes (e.g. presence or absence of a disorder, or categories representing levels of severity of a phenotype) are not normally distributed, and cannot be analyzed the same way. However, they may reflect a categorization of an underlying normally distributed trait. In this situation, a liability threshold model (Falconer, 1965) is often used. A threshold model assumes that the categories of a variable reflect an imprecise measurement of an underlying normal distribution of liability with a mean of zero and a variance of one. One or more thresholds (expressed as *Z*-scores) divide this distribution into discrete classes (e.g. *affected* vs. *unaffected* for a disease phenotype, or *no symptoms/mild/moderate/severe* for a trait measured on a continuous scale, such as a neuroticism or depression score). The area under the curve between two thresholds represents the proportion of cases within a category (Figure 2.2). The resemblance of relatives (e.g. twins) is expressed as tetrachoric or polychoric correlations, which represent the correlation of relatives on the liability dimension.

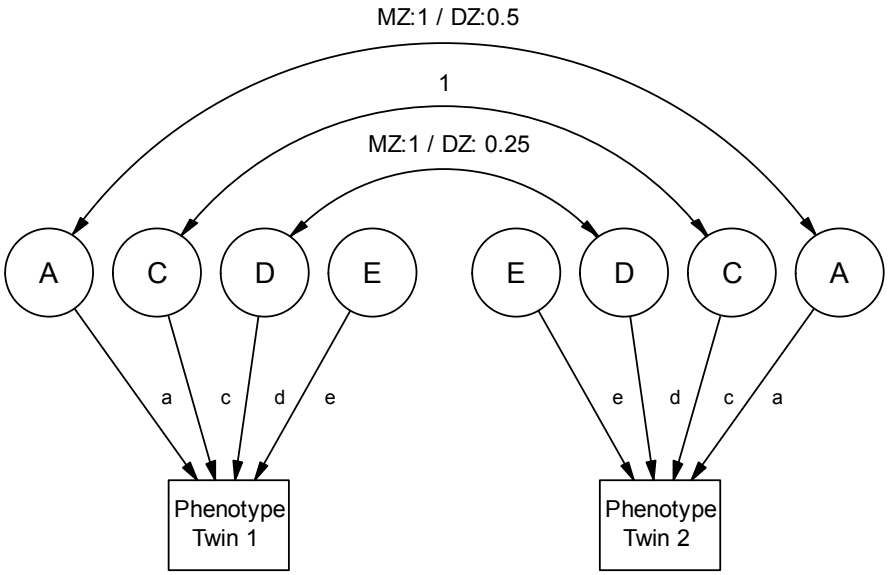


Figure 2.1

Univariate twin model. The path diagram shows the A, C, D and E factors for a twin pair, and the correlations between each of the factors for MZ and DZ twins. Following the tracing rules of path analysis (Wright, 1934), the phenotypic variance explained by each component is calculated as the squared path coefficient: the genetic variance for an individual is calculated as a^2, the shared environmental variance equals c^2, etc. The total variance is derived by summing the variance explained by the individual components: $a^2 + c^2 + d^2 + e^2$. The covariance between twins is calculated by tracing the path from twin 1, through the double-headed arrow, to twin 2. For instance, the genetic covariance between MZ twins equals $a \cdot 1 \cdot a = a^2$, whereas for DZ twins it equals $a \cdot 0.5 \cdot a = 0.5a^2$. The total covariance is calculated by adding up all paths contributing to the covariance (i.e. all paths which connect the two twins), which is $a^2 + c^2 + d^2$ for an MZ pair and $0.5a^2 + c^2 + 0.25d^2$ for a DZ pair. Note that when only data from twins reared together are available, it is not possible to estimate C and D at the same time, because there is not enough information; an ACDE model is not identified. Therefore, the twin correlations are used to decide whether an ACE or an ADE model is more plausible.

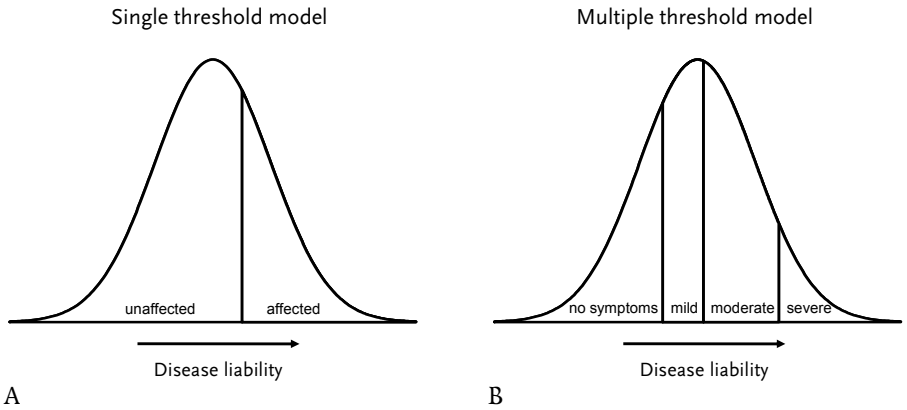


FIGURE 2.2

Threshold models. In both cases a normal distribution of liability underlies the observed phenotypes, which have been categorized into discrete classes. **A:** single threshold model; this represents a disease phenotype with affected and unaffected individuals. **B:** multiple threshold model; in this case an ordinal variable with categories corresponding to different levels of severity, in this case ranging from no symptoms, via mild and moderate, to a severe phenotype.

EXTENSIONS OF THE CLASSICAL TWIN MODEL

The classical twin design can be extended to also include data from siblings, parents and spouses. The genetic similarity between non-twin sibling pairs is the same as the resemblance between DZ twins, i.e. on average 50% of the segregating genes. Adding data from one or more non-twin siblings to the model (often referred to as an extended twin design) results in a substantial increase in power to detect genetic and shared environmental effects (e.g., Posthuma & Boomsma, 2000).

The similarity between parents and children is 50% for additive genetic effects but 0% for dominance; since dominance reflects an interaction between two alleles at the same locus, to share these effects two individuals have to share both alleles. However, parents by definition transmit only one allele to their children.

Data from parents and spouses can be used to account for the effects of parental influence (i.e. cultural transmission) and assortative mating (i.e. phenotypic correlations between spouses; Fulker, 1982). An example of this method can be found in e.g. Distel et al. (2009), who investigated whether

cultural transmission from parents to offspring had an effect on borderline personality features. They found that cultural transmission did not play a role; however, there was some evidence for assortative mating, although this explained only a small amount of the variance in the trait.

Multivariate models

A useful extension of the models described above is to analyze multiple traits simultaneously. Bivariate or multivariate models can be used to quantify the genetic and environmental overlap in correlated traits, and explore the etiology of the association (or comorbidity) between traits. For example, it is possible to test whether the same genes affect different correlated traits, or whether a similar environment is responsible for the correlation.

In addition to the MZ and DZ twin correlations, a multivariate model also includes the phenotypic correlation between traits (within a person), and the cross-twin cross-trait correlation (the correlation between trait 1 in twin 1 and trait 2 in twin 2). The function of the cross-twin cross-trait correlations is similar to that of the regular twin correlations in a univariate model: if the cross-twin cross-trait correlation is higher in MZ than in DZ twins, this indicates the two traits share a genetic component, in other words, there is a genetic correlation between them. Shared and non-shared environmental correlations are calculated similarly. Figure 2.3 shows an example of a bivariate ACE model. The cross-twin cross-trait correlations are modeled by adding the cross-paths a_{21}, c_{21} and e_{21}. If the a_{21} path is significant, this implies that a genetic correlation is present, and similarly, significance of c_{21} and e_{21} indicates shared and non-shared environmental correlations, respectively. For instance, following the tracing rules of path analysis (Wright, 1934), the genetic covariance between phenotype 1 in twin 1 and phenotype 2 in twin 2 in DZ twins is given by $a_{11} \cdot 0.5 \cdot a_{21}$.

An example of a bivariate twin analysis is described in Chapter 7. In this analysis, the relationship between migraine and depression was investigated, to test the hypothesis that the often-reported comorbidity of these disorders is due to a shared underlying genetic factor. The phenotypic correlation between the two traits was estimated at .28. Most of the shared variance (54%) was explained by genetic factors, the remaining variance was due to non-shared environment. There was a significant genetic correlation between the traits (r = .30). Thus, it can be concluded that migraine and depression are in part influenced by the same genetic and non-shared environmental factors, but that the proportion of variance explained by this relationship is modest.

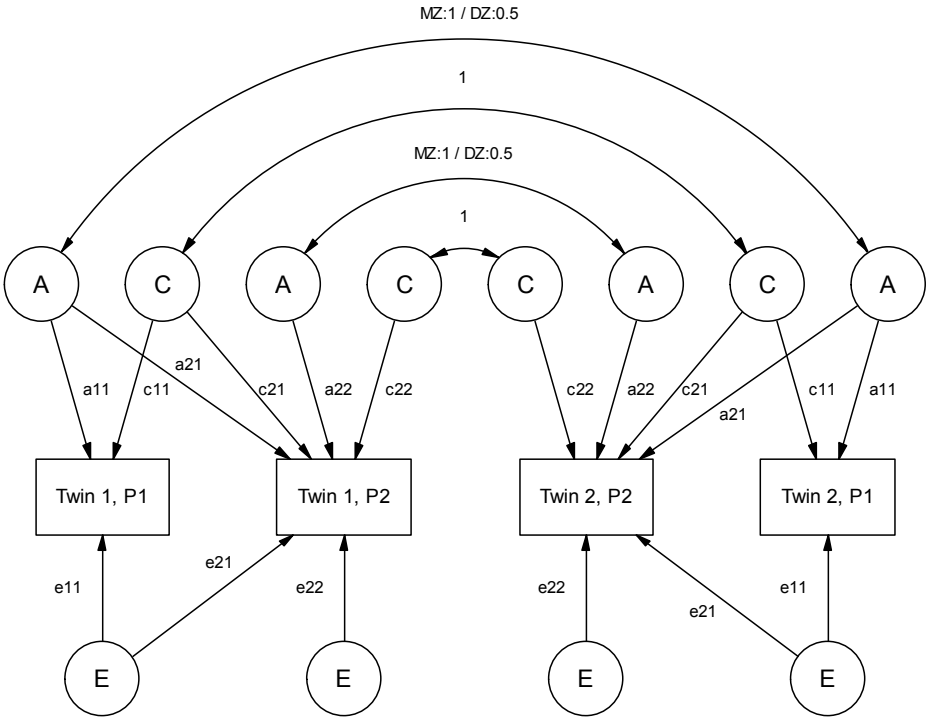


Figure 2.3

Example of a bivariate twin model: a bivariate ACE model, with two twins and two phenotypes (P1 and P2).

Another application is the extension to longitudinal models. By measuring correlations between repeated measures at different time points, it can be determined whether stability over time is due to genetic or environmental factors. An example can be found in Bartels et al. (2004), who investigated the contribution of genes and environment to stability in internalizing and externalizing problem behavior in children aged 3 to 12 years old. They found that genetic factors were responsible for both stability and change over time, while a common set of shared environmental factors mostly accounted for stability in problem behaviour across different ages. Non-shared environment played only a modest role in explaining stability or change in problem behaviour.

Sex by genotype and age by genotype interaction

The influence of genetic and environmental factors may differ for males and females. Therefore it may be useful to first test a full model in which all parameter estimates are different for the two sexes, and then test whether the estimates for males and females can be constrained to be equal. In many situations means or thresholds have to be modeled separately for males and females, for instance because a trait (e.g. migraine or depression) is more prevalent in women. Apart from that, several different hypotheses can be tested:

1. The variance components (i.e. V_A, V_D, V_C and V_E) are the same for males and females.
2. The variance components are proportionally the same in males and females, but in one sex the total trait variance is larger. A way to model this is by constraining all variance components in one sex to be a scalar multiple of the variance components in the other sex.
3. The variance components differ, for instance when a trait is more heritable in one sex than in the other. In this case, the variance components have to be estimated separately for men and women. Note that a decrease in heritability may arise for different reasons: the genetic variance can be the same in the two sexes, but the environmental variance could be larger in men than in women. Since heritability is expressed as a ratio (genetic variance over total variance) this would lead to a lower heritability estimate in men.

To test which of these models fits the data best, one starts with a full model in which all parameters are estimated separately for males and females. Then, by constraining the parameters step by step, it is tested whether parameter estimates differ significantly between men and women. This same method can be applied when data are available for different age groups (e.g. adolescents vs. adults), to test whether estimates of heritability differ depending on age. The actual implementation of the model depends on whether data have been collected in a cross-sectional design in subjects of different ages, or in a longitudinal study in which the same subjects are measured repeatedly across time.

Finally, when data from DZ opposite sex (DOS) twins are available, it is possible, in addition to quantitative differences, to also test whether qualitative sex differences are present (i.e. whether different genes affect the trait in males and females). This is tested by estimating the correlation between the latent

genetic factors in DOS twins, while this correlation remains fixed at 0.5 in the same sex pairs. If the correlation in DOS twins is significantly lower than 0.5, this is an indication that the genetic factors affecting males and females are (partly) different. It is also possible that different environmental factors influence a trait in men and women. In this case the correlation between the C factors (see Figure 2.1) would be estimated in DOS twin pairs (or in opposite-sex siblings). It is not possible to estimate the correlations for genetic and shared environmental factors simultaneously using a classical twin design, as there is only one data point available that is informative for this test.

Genotype by environment interaction

The expression of genes may also depend on environmental factors - sometimes referred to as moderators. For example, the expression of the genotype may be more clearly seen in a permissive environment. An interesting case of gene-environment interaction (GxE) was found in a study by Boomsma et al. (1999), who observed that a religious upbringing reduced the influence of genetic factors on disinhibition, one of the dimensions of the Sensation Seeking Scales. In a study of female twins Heath et al. (1998) found that being in a marriage-like relationship served as a protective factor by reducing the impact of a genetic liability to depression.

When it is known which genes influence a particular phenotype it is also possible to test for interaction of the environment with a specific gene variant. In a famous study, Caspi et al. (2003) investigated the association between the serotonin transporter gene and depression in individuals who had experienced stressful life events and individuals who had not. It was found that stressful life events were associated with depression, but only in individuals who carried at least one copy of the short allele of the serotonin transporter gene. The strongest effect was observed in individuals who carried two copies of the short allele, while the effect was non-significant in carriers of two long alleles. As spectacular as these results were, these days it is thought they may have been chance findings, since few studies since have succeeded in reproducing them. A large meta-analysis of the many replication studies failed to show significant evidence of either a main effect of the serotonin transporter gene or an interaction between this gene and stressful life events (Risch et al., 2009).

Part 2: Gene-finding

Once it has been established that a trait is heritable, the next step is to find the genes involved. The two primary statistical methods for gene-finding are linkage and association. Unlike the methods described above, linkage and association require the collection of DNA samples and the measurement of genotypes.

Linkage analysis is a method that localizes regions possibly influencing the trait of interest by using pedigree information. In short, the objective is to determine whether relatives who are phenotypically similar, are also genotypically similar in a particular region of the genome. If this is the case, this region may harbour a gene involved in the trait of interest. Linkage is based on the principle that two loci that are physically close together (e.g. an observed fragment of DNA and an unobserved disease locus) are more likely to be co-inherited. How this works will be discussed in more detail below. Because the information in a linkage study comes from the pedigree structure, it is necessary to collect family data.

Association analysis can go one step further: not only can the location of the involved regions be determined but also which genetic variant (allele) is associated with the phenotype. In other words: do individuals with a certain phenotype have a different frequency of allele X than individuals who do not have this phenotype? This can be tested with a straightforward chi-squared or regression test. Association studies have a higher resolution than linkage studies and have often been used to follow up promising linkage results. As we will see, using family data has certain advantages; however, association studies can also be performed using data from unrelated individuals.

Markers

Because - due to technical and financial limitations - it is currently not feasible to characterize the entire human DNA sequence in large numbers of individuals, gene-finding studies rely on markers. Markers are genetic variants (also called polymorphisms) with a known location which can be used as indicators of the approximate location of the real, usually unmeasured locus of interest. When we say an individual is genotyped for a linkage or association study, this means their DNA is characterized at a selected number of marker loci, either in a specific region (in candidate gene studies), or throughout the genome (in genome-wide studies).

Several types of markers are used in gene-finding studies. Single nucleotide polymorphisms (SNPs) are single base pairs with two variants (e.g. some individuals have an A, others have a C). Theoretically (if single base pair mutations have occurred multiple times at the same locus) there can be 4 variants (A, C, T and G), but for practical reasons only SNPs with two variants are selected for gene-finding studies. Microsattellites are sequence length polymorphisms that consist of a varying number of repeats of a short (usually 1-4 bp) sequence of DNA, e.g. 'CACACACACACACA'. A third and more recently recognised type of polymorphism is the copy number variant (CNV). CNVs are DNA fragments ranging from kilobases (Kb) to even megabases (Mb) in size, of which different numbers of copies are present in different individuals.

Parametric linkage

Broadly speaking, two types of linkage analysis can be distinguished: parametric and nonparametric linkage. Parametric (or model-based) linkage requires the specification of a genetic model, i.e. allele frequencies and penetrances (3 parameters specifying the probability that an individual expresses the phenotype given 0, 1 or 2 copies of the risk allele) Genotype and phenotype data from multiple generations are required to perform this type of analysis.

An important concept in parametric linkage analysis is the recombination fraction. Recombination occurs when during meiosis the maternal and paternal chromosome cross over, break and rejoin, resulting in gametes with chromosomes that are a combination of the maternal and paternal chromosome.

The recombination fraction, used in linkage analysis, is the probability that the alleles at two loci are recombinant (i.e. an odd number of recombination events has occurred between them). This depends on the distance between the loci. When two loci are located on different chromosomes, or on the same chromosome but far apart, the probability of the individual being a recombinant is around 50%, i.e. the recombination fraction (θ) is 0.5. The smaller the distance between the two loci, the lower the probability of a recombination event between them, and the lower θ will be, with $\theta = 0$ indicating perfect linkage.

To test for linkage, a genetic model is assumed, and the likelihood of the observed pedigree data under the alternative hypothesis of linkage ($\theta < 0.5$) is compared to the likelihood under the null hypothesis of no linkage ($\theta = 0.5$) between the measured marker locus and the hypothetical trait locus. The result of this test is expressed as the logarithm of odds, called the LOD score. The

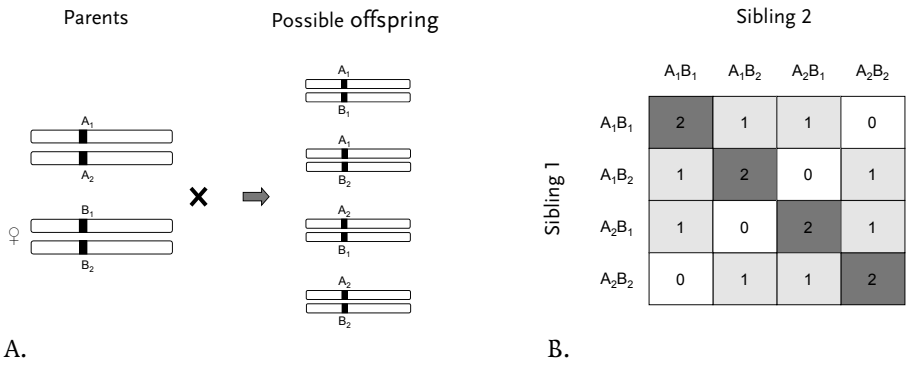
higher the LOD score, the stronger the evidence for linkage. A detailed discussion of parametric linkage methods can be found in Ott (1999).

Parametric linkage is most suited for traits that are influenced by a single gene and follow a relatively simple pattern of inheritance, because in this situation it is relatively easy to specify a genetic model. A good example of a successful parametric linkage study is described by Joutel et al. (1993), who used data from two large multigenerational families to map the first locus for familial hemiplegic migraine (the FHM1 locus) to chromosome 19. A few years later, Ophoff et al. (1996) identified several mutations in a gene in this area *(CACNA1A)* which caused the FHM phenotype. However, for many behavioral and psychological traits, specifying the correct genetic model is not straightforward.

Nonparametric (model-free) linkage

Most behavioral and psychological phenotypes are complex, i.e. they are influenced by many genes that each have a small effect. In this case it is difficult to specify a genetic model. Therefore, complex traits are usually analysed using non-parametric (also called model-free) linkage techniques. The non-parametric approach does not require the specification of a genetic model. In short, in non-parametric linkage, it is tested whether relatives with similar phenotypes also have similar genotypes. Genotypic similarity is expressed in a measure called identity by descent (IBD). Two alleles are said to be IBD if they not only have the same DNA sequence (referred to as identity by state, or IBS), but were also inherited from the same ancestor. Because there are two alleles for each locus, a pair of individuals can share 0, 1 or 2 alleles IBD. The expected probabilities for these values are ¼, ½ and ¼, respectively (Figure 2.4).

To test for linkage, the IBD values for all pairs of related individuals in the sample are estimated. For IBD estimation, the availability of parental genotypes greatly increases the accuracy of the estimates. For this reason, parental genotype data are used in linkage analysis, even when the actual LOD scores are based on data from siblings only.



A. B.

FIGURE 2.4

Possible allele combinations and identity by descent. **A:** all possible genotypes for the offspring of two parents with genotypes A_1A_2 and B_1B_2. There are four possible genotypes for the offspring which all occur with equal probability of ¼. **B**: the IBD value for all possible sibling pairs resulting from the mating depicted in A. An IBD value of 0 or 2 occurs in 4 out of 16 possibilities. Thus the probabilities are ¼, ½ and ¼ for IBD 0, 1 and 2, respectively. In this example, where both parents are heterozygotes, the IBD values of the offspring can easily be determined. However, in many situations there is insufficient information to know the IBD status with certainty. In this case IBD probabilities have to be estimated.

Several algorithms have been developed for the estimation of IBD values. The Elston-Stewart algorithm (Elston & Stewart, 1971) is suited for analysis of very large pedigrees but only for a limited number of markers at a time, because the complexity of the calculations increases exponentially with the number of markers. The Lander-Green algorithm (Lander & Green, 1987) is better suited to handle the large numbers of markers included in most modern linkage studies, but is limited to smaller pedigrees. A useful discussion of IBD estimation can be found in, e.g., Ferreira (2004).

Haseman-Elston regression

One of the first non-parametric linkage methods based on IBD estimation was introduced by Haseman & Elston (1972). This method is now known as Haseman-Elston (HE) regression. The idea was to take the squared difference in trait values for each sibling pair and regress it on the estimated IBD values at a given marker locus. There is evidence for linkage when high IBD values are

associated with strong phenotypic similarity (i.e. small squared trait differences). Thus, a significant negative regression slope indicates the presence of linkage. A drawback of HE regression is that fairly large samples are needed for sufficient statistical power to detect linkage. One method to increase power is by selecting only the most extreme cases from a population (Carey & Williamson, 1991; Dolan & Boomsma, 1998). This is possible because HE regression has the advantage that it does not rely on assumptions about the trait distribution.

Several extensions to HE regression have been proposed through the years, which improve power by using not only the squared trait differences but also the squared trait sum (e.g., Sham et al., 2002).

Variance components linkage

A non-parametric linkage method developed in the 1990's is based on variance components (VC; e.g., Almasy & Blangero, 1998; Amos, 1994). VC linkage is based on an approach similar to that described in the section about heritability estimation. In addition to the genetic and environmental components A, C, D and E, we can model the effect of a specific QTL (Q), using IBD estimations. Figure 2.5 shows a model that incorporates A (background genetic effects), Q (QTL effect) and E (environment). The correlation between the QTL factors of DZ twins and siblings equals the estimated proportion of alleles IBD, which is referred to as $\hat{\pi}$ ('pi-hat').

To test whether there is significant linkage at a certain locus the path coefficients for the Q-factor (q) are constrained to be zero. A significant deterioration of the model fit is taken as evidence for linkage. This procedure is repeated for all loci and significance levels should be adjusted accordingly. The advantage of VC linkage is that, unlike HE regression, it can be used with any type of pedigree, and it is generally more powerful. An important disadvantage, however, is its reliance on the assumption of normality of the trait distribution. Hence, the analysis of data from selected samples with variance components linkage is more involved than when HE regression is used.

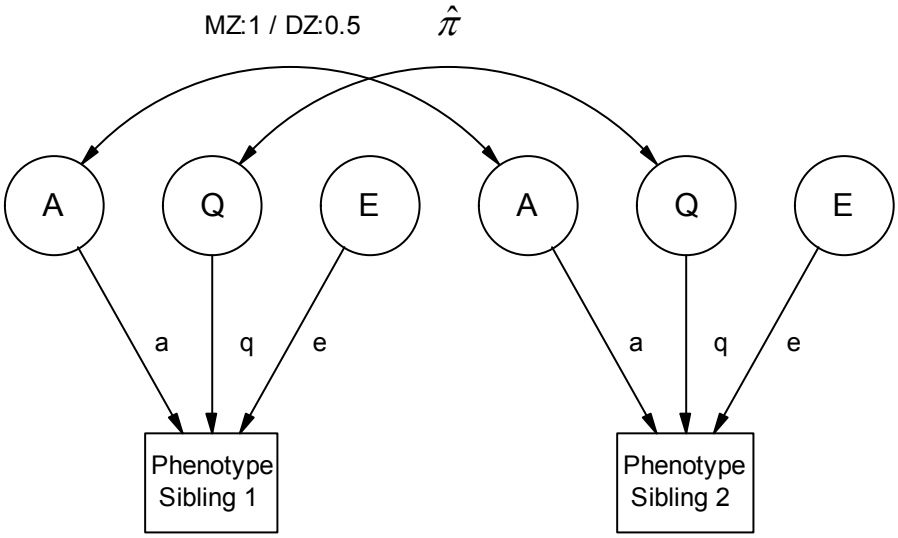


Figure 2.5

AQE model to test for linkage using a variance components approach. The correlation between the QTL effects for sibling 1 and sibling 2 equals $\hat{\pi}$ (= IBD/2), whereas the correlation between the background genetic factors of siblings or DZ twins is .5. If the mode of inheritance of the trait is largely unknown, the remaining familial variance that cannot be attributed to Q can also be modelled as simply 'familial'. If data on MZ twins are also available the familial variance can be decomposed into A and C. Note that MZ twins do not contribute any information to detect linkage (as they are perfectly correlated for all QTLs).

The affected sib pair method

For disease phenotypes (i.e. affected vs. unaffected) a commonly used linkage method is the affected sib-pair (ASP) test. In an ASP design, it is tested whether sibling pairs who are both affected for a disorder share more alleles IBD than expected in the absence of linkage (in which case the distribution should be roughly ¼, ½, ¼ for IBD values of 0, 1 and 2, respectively).

As mentioned earlier, parametric linkage was successfully used to map a gene for familial hemiplegic migraine, which is a monogenic form of migraine. Common migraine, however (i.e. MO and MA) is polygenic in nature. Therefore, nonparametric methods are more suited to investigate this disorder. Chapter 8

of this thesis describes a study in which an affected sib-pair approach was used to analyse migraine in a sample of Dutch twins and their parents and singleton siblings. Suggestive linkage was detected on chromosomes 1, 13 and 20, and a previous finding by Nyholt et al. (2005) on chromosome 5 was replicated.

The multiple testing problem (1)

These days linkage is usually performed genome-wide, in an exploratory fashion. Because in a genome-wide linkage study several hundreds of markers are tested simultaneously, a multiple testing burden is inevitable. Therefore, stringent significance thresholds have to be applied. Based on a simulation study, Lander & Kruglyak (1995) proposed using a LOD score of 3.6 to indicate significant linkage, which corresponds to a p-value of 2×10^{-5} and should be roughly equivalent to a genome-wide significance level of 5%. This has become a widely used threshold to define significance in linkage studies. Alternatively, permutation or simulation approaches using the observed data can be used to determine empirical p-values. This has the advantage that no assumptions need to be made about the null distribution of the linkage statistic.

Association

In an association study, it is tested whether a particular allele or genotype is more prevalent in individuals with a certain phenotype. For instance, do individuals with allele C at a given SNP have a higher depression score than individuals with allele A?

Association analysis can be performed in unrelated individuals or in family-based samples. Studies in unrelated samples are often set up as case-control studies: allele or genotype frequencies are compared between a selection of cases and a group of matched controls. It is also possible to test for association with a continuous phenotype: in this case mean trait values are compared between individuals with different genotypes. The advantage of case-control association studies is the relative ease of collecting samples and the straightforward statistical tests that can be used. The disadvantage, however, is that the presence of an underlying population substructure can lead to spurious results, a phenomenon referred to as 'population stratification'. This phenomenon is illustrated in a famous paper by Hamer & Sirota (2000). The paper describes a hypothetical study in a student population consisting of Caucasian and Asian subjects, in which a gene is identified for eating with chopsticks. However, this is not a true association, but the result of the fact that, for all sorts of reasons, allele frequencies can differ between the two

populations. The two populations happen to also differ in terms of eating with chopsticks, which is entirely culturally determined. However, from the association analysis it falsely appears that the gene has something to do with the chopsticks.

One way to deal with stratification issues is by using a family-based association test, such as the haplotype relative risk (Falk & Rubinstein, 1987; Terwilliger & Ott, 1992), or the transmission disequilibrium test (Spielman et al., 1993), which use data from heterozygous parents and affected children to determine which parental alleles are transmitted to an affected child and which are not. Thus, the non-transmitted alleles serve as 'internal' control genotypes, which eliminates the need for external controls and the risk of stratification issues. The disadvantage is that family-based samples are more difficult to collect, because both parents have to be present (which can be particularly challenging for late-onset phenotypes such as Alzheimer's disease or ageing).

An alternative approach, suitable for quantitative traits, was developed by Fulker et al. (1999). With this method, which uses data from sibling pairs, the effects of genes on phenotypic means are partitioned into a between and within-family component. A within-family association test is not affected by population stratification because siblings within a family belong to the same stratum. Thus, it is tested whether an allele is associated with the phenotype in siblings within the same family, whether they are associated in siblings from different families, and whether the effect size of these tests is the same. If the gene effect is different between families than within families, there is evidence for population stratification. If, however, the within-family effect alone is significant, regardless of the between-family effect, this means there is still evidence for a true association effect, not due to population substructure (Fulker et al., 1999). This method has been implemented in the QTDT program (Abecasis et al., 2000).

In situations where unrelated individuals are used for association analysis, other methods are available to assess and control for population stratification, such as calculating the genomic inflation factor and applying genomic control. These will be discussed in more detail later.

Linkage Disequilibrium

An important concept in association studies is the phenomenon of linkage disequilibrium (LD). When two loci are in linkage equilibrium, the genotype at locus 1 is independent of the genotype at locus 2. This is usually the case when loci are on different chromosomes or far apart on the same chromosome.

However, if two loci are close together and over generations little recombination has taken place between them, the genotype at locus 1 may be associated with the genotype at locus 2. Therefore, when an association is found, this can be due to either direct or indirect association. In the case of direct association, the association signal comes from the actual causal variant. An association that arises because the marker is in LD with the causal variant, it is called indirect association.

Candidate gene studies vs. genome-wide association

Until recently, association studies focused on smaller candidate regions. Based on existing knowledge (e.g. theories about biochemical pathways or evidence from linkage studies), candidate genes were identified and genotyping was restricted to the region of interest. Good examples are association studies of the serotonin receptor and transporter genes in both depression and migraine studies. Both conditions are often successfully treated with drugs that interact with the serotonergic system (selective serotonin reuptake inhibitors [SSRIs] and triptans, respectively), suggesting a possible causal involvement of serotonin in the etiology of the disorders. However, in spite of the large number of studies conducted, it has proven difficult to unequivocally demonstrate a role of serotonin receptor or transporter genes in the pathogenesis of depression (Anguelova et al., 2003; Risch et al., 2009). A similar conclusion can be drawn for migraine (Colson et al., 2007). Although there is limited evidence for a possible role of certain serotonin-related genes in migraine and depression, the majority of candidate-gene association studies have returned negative results. This may be illustrative of the main weakness of the candidate gene approach: usually our knowledge about the pathways involved is very limited, making it very difficult to determine which genes are good candidates.

Due to the availability of faster and cheaper genotyping techniques it has now become feasible to genotype enough markers (from 300.000 up to 1 million) to cover most of the common variation in the entire human genome, and perform genome-wide association analysis (GWA). Several companies (e.g. Illumina, Affymetrix) produce pre-designed SNP chips that include a selection of carefully chosen 'tag SNPs'. Tag SNPs are selected based on LD patterns, in such a way that a minimum number of SNPs captures a maximum amount of genetic variation in the population it is designed for. In contrast with candidate gene studies, a GWA study is exploratory in nature; no prior hypothesis about the location of causative genes is necessary. Indeed, many of the associations identified through GWA studies to date were not previously regarded as

candidates, which demonstrates the use of exploratory gene-finding studies (Manolio & Collins, 2009).

The multiple testing problem (2)

Due to the large numbers of markers used, the multiple testing burden in a GWA study is even larger than in a linkage study, which makes it crucially important to use appropriate significance thresholds. The exact multiple testing burden depends on the set of SNPs included in the study and on the population studied. For instance, African populations are known to have less LD and more SNPs, and therefore the multiple testing burden will be higher than in a European population. Several authors have proposed cut-off values for significance in GWA studies. Pe'er et al. (2008) recommended multiplying the nominal p-value by the genome-wide testing burden, which, according to their calculations is roughly half a million tests when all common SNPs are tested in a European (Hapmap CEU) population. To obtain a genome-wide significance level of 5%, this means a nominal threshold of $P = 1 \times 10^{-7}$ should be used. Dudbridge & Gusnanto (2008) used a permutation approach to estimate the genome-wide significance threshold in the UK Caucasian population. They estimated that genome-wide significance at the 5% level corresponded to a nominal P-value of 7.2×10^{-8}, and state that any P-value below 5×10^{-8} can be considered "convincingly significant".

It should be noted that even the use of strict significance thresholds has not been able to avoid that many candidate-gene association studies have produced results that could not be replicated, possibly because many of them were false positive findings (Hirschhorn et al., 2002). Since the credibility of a finding increases considerably when it is replicated in multiple independent samples, it is now a common requirement for GWA studies that results be replicated internally (i.e. in an independent sample described in the same study), in order to be published.

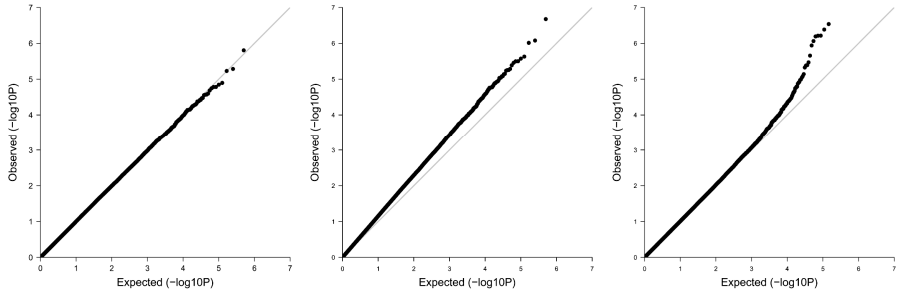


FIGURE 2.6

Examples of Q-Q plots. The expected distribution of p-values (x-axis) is plotted against the observed distribution (y-axis). For convenience, p-values in a GWA study are often shown on a logarithmic scale, i.e. $-\log_{10}(P)$. **A:** The observations closely follow the expected distribution (shown in grey), indicating there is probably no association and no inflation of the distribution either. **B:** Inflation across the whole distribution, which may indicate population stratification. **C:** An excess of small p-values in the tail of the distribution, possibly indicating some true associations.

GENOMIC INFLATION

As mentioned before, population substructure is a factor that can lead to spurious results in an association study. Therefore, in the design of a GWA study it is important to carefully select the individuals to be genotyped to avoid problems related to stratification within the sample. Once the data have been collected, it is common practice to run some quality control checks to scan for potential problems. A good way to get a first impression of the results is by creating a quantile-quantile plot (also called Q-Q plot). In a Q-Q plot, the expected distribution of p-values is plotted against the observed distribution (Figure 2.6). Under the assumption of no true association signals, this should result in a straight diagonal line. An excess of small p-values, resulting in a deviation in the tail of the distribution, may indicate true association signals. However, if the observed findings are inflated (i.e. show an excess of small p-values) across the entire distribution, this may indicate population stratification (McCarthy et al., 2008). The extent to which the distribution is inflated can be expressed in a statistic called the genomic inflation factor, λ (lambda), which is calculated as the median χ^2 of the observed distribution, divided by the median

χ^2 of the expected distribution. Ideally, λ should approach a value of 1. Based on the value of λ, the test statistic can be rescaled to correct it for inflation. This procedure is called genomic control (Devlin & Roeder, 1999).

Meta-analysis

GWA studies have shown to be effective and associations have been successfully identified for quite a number of human traits, such as Crohn's disease (Barrett et al., 2008), type 2 diabetes (Zeggini et al., 2008), bipolar disorder (Ferreira et al., 2008) and obesity (Lindgren et al., 2009). However, it has become clear that for most complex traits the observed effects are small and therefore very large samples are needed. Visscher (2008) estimated that to detect a variant that explains 0.1-0.5% of the variance in a quantitative trait (which may be a realistic effect size for genes affecting complex traits), tens of thousands of individuals are necessary for sufficient power. Since no single study has the budget to collect these enormous amounts of data, it is a necessity to combine GWA studies. For this reason, large consortia have been formed in recent years to enable meta-analyses of GWA results (e.g., Barrett et al., 2008; Lindgren et al., 2009; Zeggini et al., 2008). In a meta-analysis, the results of multiple individual studies are combined into one overall test statistic. Two types of meta-analysis can be distinguished: methods that assume fixed effects and those that assume random effects. Fixed effects methods assume there is one common effect in all studies (homogeneity), and that between-study variability is due to chance. Two frequently used fixed-effects methods are the inverse-variance weighted method and the pooled *Z*-score method. The inverse-variance weighted method pools the betas and standard errors from all studies, weighting each study by the inverse of the variance of beta. The outcome is an effect estimate for each SNP, pooled across all studies. This method is most suitable when the phenotype is measured on the same scale in all studies, so that beta can be interpreted the same way for all samples. The pooled *Z*-score method does not pool effect sizes but *Z*-scores, weighted by sample size. It provides information on the direction and significance of the pooled effect, but not about the effect size. This method is more appropriate when the phenotype is not measured on the same scale across studies and hence the effect sizes are not directly comparable.

In cases where different genetic effects are expected across studies (heterogeneity), for instance because the populations have a different genetic background, random effects methods are more appropriate. Various metrics are available to assess the presence of heterogeneity, such as Cochran's Q statistic or I^2 (Kavvoura & Ioannidis, 2008). The main drawback of random effects

methods, however, is that they are more conservative and thus have low power compared to fixed effects models. A useful practical guideline for meta-analysis of genome-wide association studies is provided by de Bakker et al. (2008).

HapMap & imputation

One problem in meta-analysis of GWA results is the fact that different studies use different SNP chips, which tend to be largely non-overlapping. As a consequence, the number of SNPs genotyped in all studies is limited. This can be overcome by imputing the genotypes of SNPs that were not measured, using data generated by the International HapMap project (2003; www.hapmap.org). The HapMap project was launched in 2002 with the purpose of creating a 'haplotype map' of the human genome that describes common patterns of genetic sequence variation. In phase I and II of the project 270 individuals from 4 populations (European, Nigerian, Japanese and Han Chinese) were genotyped to obtain information on more than 3.1 million SNPs. In phase III the project was expanded to include data from another 7 populations.

The HapMap data can be used to infer a missing genotype at one marker from available genotypes at other markers. This is possible because, due to the presence of LD, only a limited number of haplotypes frequently occur in the population, even though theoretically much more variation would be possible. To infer missing genotypes, a genotyped individual is compared to a HapMap reference sample. Because the LD structure in the HapMap sample is known it can be determined, given the available genotypes, what the most likely genotype is for the missing SNP. For instance, if all reference individuals with a certain haplotype have a C allele at SNP *X*, and SNP *X* is in high LD with this haplotype, an individual with the same haplotype but a missing genotype at SNP *X* is highly likely to also have a C allele.

Clearly there is some uncertainty involved in determining the most likely genotype for a missing SNP. For this reason, imputation programs calculate a probability for each possible genotype, and provide a quality measure that indicates how reliable the imputation is for each SNP, so that in the analysis stage, the researcher can decide to remove SNPs that were poorly imputed. In addition, the probability scores for the different genotypes can be used to account for the uncertainty of the imputations.

One limitation of the HapMap is that it covers only common variation. Therefore, if a trait is primarily influenced by rare alleles, associations will not be detected using the HapMap SNPs. The aim of the more recently started 1000 Genomes Project (www.1000genomes.org) is to provide coverage of the rarer

variants as well, and to provide a more detailed map of the human genome. In order to do this, whole genomes of approximately 1200 individuals will be sequenced (i.e. their entire DNA sequence will be determined).

BOX: Genetics software on the internet

VARIOUS TYPES OF ANALYSIS:

Merlin (Abecasis et al., 2002): http://www.sph.umich.edu/csg/abecasis/merlin/
For various types of parametric and non-parametric linkage, and association analysis in family data.

Mx (Neale et al., 2003): http://www.vcu.edu/mx/
Package for structural equation modelling, especially suitable for twin modelling and variance components linkage analysis.

GENOME-WIDE ASSOCIATION:

Plink (Purcell et al., 2007): http://pngu.mgh.harvard.edu/~purcell/plink/
GenABEL (R package) - http://mga.bionet.nsc.ru/~yurii/ABEL/GenABEL/

IMPUTATION:

MACH (Li & Abecasis, 2006): http://www.sph.umich.edu/csg/abecasis/mach/
IMPUTE (Marchini et al., 2007): http://mathgen.stats.ox.ac.uk/impute/impute.html

ANALYSIS OF IMPUTED DATA:

SNPTEST (Marchini et al., 2007):
http://www.stats.ox.ac.uk/~marchini/software/gwas/snptest.html
ProbABEL: http://mga.bionet.nsc.ru/~yurii/ABEL/

META-ANALYSIS:

METAL: http://www.sph.umich.edu/csg/abecasis/metal/
MetABEL (R package): http://mga.bionet.nsc.ru/~yurii/ABEL/

OTHER USEFUL WEBSITES:

Shaun Purcell's behavioral genetic interactive modules:
http://pngu.mgh.harvard.edu/~purcell/bgim/
Greg Carey's interactive learning exercises on behavior genetics:
http://psych.colorado.edu/~carey/hgss/hgssapplets/hgssapplets.htm
Mx script library (example scripts for twin modelling):
http://www.psy.vu.nl/mxbib/

Part III: Beyond Gene-finding

A person's phenotype depends on more than simply the genetic code. Genes exert their effects through their products, usually proteins. For proteins to be produced, a gene has to be expressed. The main steps in gene expression are transcription and translation. During transcription, the DNA molecule serves as a template to construct an RNA copy of itself (an RNA molecule resembles DNA but contains Uracil (U) instead of Thymine (T) bases, and is single-stranded). The RNA codes for a sequence of amino acids, together forming a protein. The construction of a protein, based on the RNA code, is called translation.

The expression of genes is affected by various factors, such as epigenetic modifications (see below) and regulation by other genes or transcription factors (proteins that bind to DNA, thereby controlling the expression of genes). In this last section, we will discuss the effects of epigenetic modification on gene expression, and the use of genome-wide expression data in gene-finding studies. Finally, a closely related area of research is the study of interactions between genes in biological networks and pathways. Identifying these pathways is an important step from statistical linkage or associations to understanding the biology underlying human traits and diseases.

Epigenetics

Epigenetics is the study of heritable changes in gene expression that are unrelated to changes in the DNA sequence. Epigenetic changes are caused by chemical modifications that affect the expression of genes. There are two types of modification that cause epigenetic changes: DNA methylation and histone modification. DNA methylation is the addition of a methyl group to a cytosine base that is followed by guanine (a so-called CpG site, where the p refers to the phosphodiester bond that connects two bases). CpG sites tend to occur in large repetitive sequences which are highly methylated, or in short CpG-rich DNA stretches called CpG islands, which are mostly unmethylated. CpG islands frequently overlap with the promoter region of genes (i.e. a region close to the gene where the transcription process is initiated). It is thought that methylation affects gene expression by controlling whether or not proteins that affect transcription can bind to the DNA (Jaenisch & Bird, 2003).

The second type of alteration is the modification of histones. Histones are the proteins around which DNA molecules are wrapped. There are various types of chemical modification of histones, including methylation and modifications affecting how densely the DNA is 'packed'. A tightly packed structure of the

DNA prevents gene expression, whereas in relaxed DNA gene expression is active.

One might say that the epigenome has a lifecycle. After fertilization, most of the DNA is demethylized and a new wave of methylation occurs. This methylation pattern is inherited from parent to daughter cells during cell division, providing what might be called an 'epigenetic memory'. Later in development, tissue-specific changes in methylation occur, which aid the differentiation of different cell types. At present, not much is known about how these changes occur (Feinberg, 2008). An interesting aspect of this phenomenon is that epigenetic changes are easier to reverse than genetic mutations, which may offer possibilities for the treatment of disease with drugs (e.g., Smith et al., 2007).

An additional factor that influences methylation patterns during the lifespan is the environment. Diet, for instance, has been suggested as an environmental factor that influences epigenetic processes. Diet-mediated epigenetic effects have been implicated in a variety of conditions, such as cancer, cardiovascular disease, but also depression and other psychiatric disorders (Van den Veyver, 2002). In recent years, it has become clear that epigenetics may explain part of the differences observed in genetically identical MZ twins. These differences will be part of the non-shared environmental component in a twin study. An interesting study in MZ twins showed that twins who were older, had more different lifestyles and spent less of their lifetimes together displayed more different epigenetic profiles than younger twins who shared most of their environment and lifestyle (Fraga et al., 2005). On the other hand, Heijmans et al. (2007), who combined an epigenetic study with a classical twin design, found that most of the variation across individuals in DNA methylation at the locus they investigated *(IGF2/H19)* could be attributed to heritable factors. The influence of environmental factors did not increase with age, suggesting at least some loci are relatively unaffected by age-related changes in methylation.

Gene expression

The genome-wide study of gene expression is a rapidly developing area of research. To measure gene expression, the transcript (RNA) content of a tissue sample is analyzed to determine which genes are being transcribed and in which quantities. One application of gene expression analysis is to combine it with the regular GWA approach. In this type of study, gene transcript abundance is treated as a phenotype, and can be mapped to genomic loci, called 'expression

QTLs' (eQTLs). This approach identifies markers that are associated with the expression of a gene and is useful to identify genetic variants that regulate the expression of other genes (Gilad et al., 2008). An example of how this might work is the situation where a strong association signal is found with an area that contains no genes (a so-called gene desert), a phenomenon that is regularly observed (Manolio & Collins, 2009). This region may harbour some regulatory sequence that influences the expression of a gene located at some distance from the associated SNP. An expression study might reveal this mechanism by detecting an association between the SNP in the gene desert and the expression level of the distant gene, which would otherwise go unnoticed.

A complicating factor in the collection of expression data is that expression levels differ depending on the type of tissue. Ideally, gene expression is measured in the tissues involved in the disease or trait of interest, however, in many cases (e.g. brain disease) it is not an easy task to obtain the right tissue samples in sufficient quantities. One possible solution could be to use more easily accessible tissues as a surrogate for the tissue of interest. For instance, Sullivan et al. (2006) compared gene expression in whole blood and 16 different tissues from the central nervous system (CNS) to assess the feasibility of using whole blood samples as a surrogate for brain tissue samples. They concluded that, although imperfect, there is a correlation between CNS and whole blood gene expression (with a median around 0.5), and that in some situations the cautious use of whole blood gene expression data could be a useful proxy measure of CNS gene expression.

To investigate the feasibility of large scale expression data collection, a pilot project called the Genotype-Tissue Expression project (GTEx; http://nihroadmap.nih.gov/GTEx/) was recently announced. The aim of this project is to develop a database containing expression data from approximately 1000 donor individuals in 30 different types of tissue. These individuals will also be genotyped at high density. It is hoped that with these data a comprehensive database of human eQTLs can be developed.

Pathway analysis

Variation or disruptions in different genes can have similar phenotypic consequences if the genes are involved in the same pathway. Disruptions at different stages of a pathway might all, independently or in interaction, lead to an increased risk of disease or expression of a complex trait. Pathway-analysis investigates whether a number of genes that have been found in e.g. a genetic

association study, are more often involved in a certain biological pathway than expected by chance (Wang et al., 2007).

Studies that have employed GWA and pathway analysis have reported some promising results (Ritchie, 2009). For example, Vink et al. (2009) searched for genes that may be involved in smoking behaviour, both initiation and persistence. Genes that showed an association with smoking behaviour in multiple samples were analyzed in terms of biological function, cellular location and possible interactions of the gene products. Using this approach they identified several groups of genes of similar function which may affect smoking behaviour. Several other phenotypes have been investigated using similar approaches, including multiple sclerosis (Baranzini et al., 2009), type 1 and 2 diabetes and bipolar disorder (Torkamani et al., 2008). Many others will undoubtedly follow. This type of analysis may be an important new step towards understanding the biological mechanisms underlying a trait.

Clearly, the introduction of genome-wide SNP arrays initiated many rapid developments in the field of gene-finding, and this may only be the beginning. New approaches such as the gene network and pathway-based analyses are only just starting to be developed. Although there have been many successes, there are also plenty of challenges left, especially in terms of the management and analysis of the huge amounts of data that are available already, and the even larger amounts of new data that are currently being collected, such as whole genome sequence data. Given the promising results published in recent years, we can only expect more to come.

References

Abecasis, G. R., Cardon, L. R., & Cookson, W. O. (2000). A general test of association for quantitative traits in nuclear families. Am J Hum Genet, 66(1), 279-292.

Abecasis, G. R., Cherny, S. S., Cookson, W. O., & Cardon, L. R. (2002). Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet, 30(1), 97-101.

Akaike, H. (1987). Factor analysis and AIC. Psychometrika, 52, 317-332.

Almasy, L., & Blangero, J. (1998). Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet, 62(5), 1198-1211.

Amos, C. I. (1994). Robust variance-components approach for assessing genetic linkage in pedigrees. Am J Hum Genet, 54(3), 535-543.

Anguelova, M., Benkelfat, C., & Turecki, G. (2003). A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: I. Affective disorders. Mol Psychiatry, 8(6), 574-591.

Baranzini, S. E., Galwey, N. W., Wang, J., Khankhanian, P., Lindberg, R., Pelletier, D., et al. (2009). Pathway and network-based analysis of genome-wide association studies in multiple sclerosis. Hum Mol Genet, 18(11), 2078-2090.

Barrett, J. C., Hansoul, S., Nicolae, D. L., Cho, J. H., Duerr, R. H., Rioux, J. D., et al. (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet, 40(8), 955-962.

Bartels, M., van den Oord, E. J., Hudziak, J. J., Rietveld, M. J., van Beijsterveldt, C. E., & Boomsma, D. I. (2004). Genetic and environmental mechanisms underlying stability and change in problem behaviors at ages 3, 7, 10, and 12. Dev Psychol, 40(5), 852-867.

Boomsma, D. I., de Geus, E. J., van Baal, G. C., & Koopmans, J. R. (1999). A religious upbringing reduces the influence of genetic factors on disinhibition: evidence for interaction between genotype and environment on personality. Twin Res, 2(2), 115-125.

Browne, M. W., & Cudeck, R. (1993). Alternative ways of assessing model fit. In K. A. Bollen & J. S. Long (Eds.), Testing structural equation models (pp. 136-162). Newbury Park, CA: Sage.

Carey, G., & Williamson, J. (1991). Linkage analysis of quantitative traits: increased power by using selected samples. Am J Hum Genet, 49(4), 786-796.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., et al. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science, 301(5631), 386-389.

Colson, N. J., Fernandez, F., Lea, R. A., & Griffiths, L. R. (2007). The search for migraine genes: an overview of current knowledge. Cell Mol Life Sci, 64(3), 331-344.

De Bakker, P. I., Ferreira, M. A., Jia, X., Neale, B. M., Raychaudhuri, S., & Voight, B. F. (2008). Practical aspects of imputation-driven meta-analysis of genome-wide association studies. Hum Mol Genet, 17(R2), R122-128.

Devlin, B., & Roeder, K. (1999). Genomic control for association studies. Biometrics, 55(4), 997-1004.

Distel, M. A., Rebollo-Mesa, I., Willemsen, G., Derom, C. A., Trull, T. J., Martin, N. G., et al. (2009). Familial resemblance of borderline personality disorder features: genetic or cultural transmission? PLoS One, 4(4), e5334.

Dolan, C. V., & Boomsma, D. I. (1998). Optimal selection of sib pairs from random samples for linkage analysis of a QTL using the EDAC test. Behav Genet, 28(3), 197-206.

Dudbridge, F., & Gusnanto, A. (2008). Estimation of significance thresholds for genomewide association scans. Genet Epidemiol, 32(3), 227-234.

Elston, R. C., & Stewart, J. (1971). A general model for the genetic analysis of pedigree data. Hum Hered, 21(6), 523-542.

Falconer, D. S. (1965). The Inheritance of Liability to Certain Diseases Estimated from Incidence among Relatives. Annals of Human Genetics, 29, 51-76.

Falconer, D. S., & Mackay, T. F. (1996). Introduction to quantitative genetics (4th ed.). Harlow: Longman.

Falk, C. T., & Rubinstein, P. (1987). Haplotype relative risks: an easy reliable way to construct a proper control sample for risk calculations. Ann Hum Genet, 51(Pt 3), 227-233.

Feinberg, A. P. (2008). Epigenetics at the epicenter of modern medicine. Jama-Journal of the American Medical Association, 299(11), 1345-1350.

Ferreira, M. A. (2004). Linkage analysis: principles and methods for the analysis of human quantitative traits. Twin Res, 7(5), 513-530.

Ferreira, M. A., O'Donovan, M. C., Meng, Y. A., Jones, I. R., Ruderfer, D. M., Jones, L., et al. (2008). Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. Nat Genet, 40(9), 1056-1058.

Fischer, R. A. (1918). The correlation between relatives on the supposition of Mendelian inheritance. Transactions of the Royal Society of Edinburgh, 52, 399–433.

Fraga, M. F., Ballestar, E., Paz, M. F., Ropero, S., Setien, F., Ballestar, M. L., et al. (2005). Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci U S A, 102(30), 10604-10609.

Fulker, D. W. (1982). Extensions of the classical twn method. In B. Bonne-Tamir (Ed.), Human genetics, part A: The unfolding genome (pp. 395-406). New York: Alan R. Liss.

Fulker, D. W., Cherny, S. S., Sham, P. C., & Hewitt, J. K. (1999). Combined linkage and association sib-pair analysis for quantitative traits. Am J Hum Genet, 64(1), 259-267.

Gilad, Y., Rifkin, S. A., & Pritchard, J. K. (2008). Revealing the architecture of gene regulation: the promise of eQTL studies. Trends Genet, 24(8), 408-415.

Hamer, D., & Sirota, L. (2000). Beware the chopsticks gene. Mol Psychiatry, 5(1), 11-13.

Haseman, J. K., & Elston, R. C. (1972). The investigation of linkage between a quantitative trait and a marker locus. Behav Genet, 2(1), 3-19.

Heath, A. C., Eaves, L. J., & Martin, N. G. (1998). Interaction of marital status and genetic risk for symptoms of depression. Twin Res, 1(3), 119-122.

Heijmans, B. T., Kremer, D., Tobi, E. W., Boomsma, D. I., & Slagboom, P. E. (2007). Heritable rather than age-related environmental and stochastic factors dominate variation in DNA methylation of the human IGF2/H19 locus. Hum Mol Genet, 16(5), 547-554.

Hirschhorn, J. N., Lohmueller, K., Byrne, E., & Hirschhorn, K. (2002). A comprehensive review of genetic association studies. Genet Med, 4(2), 45-61.

International Human Genome Sequencing Consortium (2004). Finishing the euchromatic sequence of the human genome, Nature (Vol. 431, pp. 931-945).

Jaenisch, R., & Bird, A. (2003). Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet, 33 Suppl, 245-254.

Joutel, A., Bousser, M. G., Biousse, V., Labauge, P., Chabriat, H., Nibbio, A., et al. (1993). A gene for familial hemiplegic migraine maps to chromosome 19. Nat Genet, 5(1), 40-45.

Kavvoura, F. K., & Ioannidis, J. P. (2008). Methods for meta-analysis in genetic association studies: a review of their potential and pitfalls. Hum Genet, 123(1), 1-14.

Lander, E., & Kruglyak, L. (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet, 11(3), 241-247.

Lander, E. S., & Green, P. (1987). Construction of multilocus genetic linkage maps in humans. Proc Natl Acad Sci U S A, 84(8), 2363-2367.

Li, Y., & Abecasis, G. R. (2006). Mach 1.0: Rapid Haplotype Reconstruction and Missing Genotype Inference. Am J Hum Genet, S79, 2290.

Liang, F., Holt, I., Pertea, G., Karamycheva, S., Salzberg, S. L., & Quackenbush, J. (2000). Gene index analysis of the human genome estimates approximately 120,000 genes. Nat Genet, 25(2), 239-240.

Lindgren, C. M., Heid, I. M., Randall, J. C., Lamina, C., Steinthorsdottir, V., Qi, L., et al. (2009). Genome-wide association scan meta-analysis identifies three Loci influencing adiposity and fat distribution. PLoS Genet, 5(6), e1000508.

Manolio, T. A., & Collins, F. S. (2009). The HapMap and genome-wide association studies in diagnosis and therapy. Annu Rev Med, 60, 443-456.

Marchini, J., Howie, B., Myers, S., McVean, G., & Donnelly, P. (2007). A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet, 39(7), 906-913.

McCarthy, M. I., Abecasis, G. R., Cardon, L. R., Goldstein, D. B., Little, J., Ioannidis, J. P., et al. (2008). Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet, 9(5), 356-369.

Neale, M. C., Boker, S. M., Xie, G., & Maes, H. H. (2003). Mx: Statistical Modeling (6 ed.). VCU Box 900126, Richmond, VA 23298: Department of psychiatry.

Nyholt, D. R., Morley, K. I., Ferreira, M. A., Medland, S. E., Boomsma, D. I., Heath, A. C., et al. (2005). Genomewide significant linkage to migrainous headache on chromosome 5q21. Am J Hum Genet, 77(3), 500-512.

Ophoff, R. A., Terwindt, G. M., Vergouwe, M. N., van Eijk, R., Oefner, P. J., Hoffman, S. M., et al. (1996). Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell, 87(3), 543-552.

Ott, J. (1999). Analysis of human genetic linkage (3rd ed.). Baltimore MD: Johns Hopkins University Press.

Pe'er, I., Yelensky, R., Altshuler, D., & Daly, M. J. (2008). Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. Genet Epidemiol, 32(4), 381-385.

Posthuma, D., & Boomsma, D. I. (2000). A note on the statistical power in extended twin designs. Behav Genet, 30(2), 147-158.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M. A., Bender, D., et al. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet, 81(3), 559-575.

Risch, N., Herrell, R., Lehner, T., Liang, K. Y., Eaves, L., Hoh, J., et al. (2009). Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. Jama, 301(23), 2462-2471.

Ritchie, M. D. (2009). Using prior knowledge and genome-wide association to identify pathways involved in multiple sclerosis. Genome Med, 1(6), 65.

Schwarz, G. (1978). Estimating the dimension of a model. The Annals of Statistics, 6, 461-464.

Sham, P. C., Purcell, S., Cherny, S. S., & Abecasis, G. R. (2002). Powerful regression-based quantitative-trait linkage analysis of general pedigrees. Am J Hum Genet, 71(2), 238-253.

Smith, L. T., Otterson, G. A., & Plass, C. (2007). Unraveling the epigenetic code of cancer for therapy. Trends Genet, 23(9), 449-456.

Spielman, R. S., McGinnis, R. E., & Ewens, W. J. (1993). Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet, 52(3), 506-516.

Sullivan, P. F., Fan, C., & Perou, C. M. (2006). Evaluating the comparability of gene expression in blood and brain. Am J Med Genet B Neuropsychiatr Genet, 141B(3), 261-268.

Terwilliger, J. D., & Ott, J. (1992). A haplotype-based 'haplotype relative risk' approach to detecting allelic associations. Hum Hered, 42(6), 337-346.

Torkamani, A., Topol, E. J., & Schork, N. J. (2008). Pathway analysis of seven common diseases assessed by genome-wide association. Genomics, 92(5), 265-272.

Van den Veyver, I. B. (2002). Genetic effects of methylation diets. Annu Rev Nutr, 22, 255-282.

Vink, J. M., Smit, A. B., de Geus, E. J., Sullivan, P., Willemsen, G., Hottenga, J. J., et al. (2009). Genome-wide association study of smoking initiation and current smoking. Am J Hum Genet, 84(3), 367-379.

Visscher, P. M. (2008). Sizing up human height variation. Nat Genet, 40(5), 489-490.

Wang, K., Li, M., & Bucan, M. (2007). Pathway-Based Approaches for Analysis of Genomewide Association Studies. Am J Hum Genet, 81(6).

Wright, S. (1934). The method of path coefficients. Annals of Mathematical Statistics, 5, 161-215.

Zeggini, E., Scott, L. J., Saxena, R., Voight, B. F., Marchini, J. L., Hu, T., et al. (2008). Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet, 40(5), 638-645.

3

Data Collection

INTRODUCTION

The majority of the data this thesis is based on were collected by the Netherlands Twin Registry (NTR), within the framework of an ongoing longitudinal study of lifestyle, health and personality in Dutch twins, their parents, siblings, partners and children (Boomsma et al., 2006; Boomsma et al., 2002). Between 1990 and 1993, twin families were recruited by asking city councils to provide details of twins aged between 13 and 20 years of age. Later, additional recruitment was based on advertisements and a yearly newsletter, and in addition to twins, parents and siblings, recruitment was extended to also include partners and children of twins. Since 1991, adult members of the NTR have received mailed surveys every two to three years. These surveys include sections about physical and mental health, lifestyle, and personality. Table 3.1 provides a summary of the number of participants in all NTR surveys.

TABLE 3.1

Number of participants across all adult NTR surveys							
Year of survey	**1991**	**1993**	**1995**	**1997**	**2000**	**2002**	**2004**
Twins/triplets	3386	4224	3413	3231	4610	4523	5382
Siblings	0	0	1478	1517	1474	1454	1691
Parents	3045	3694	3260	5	3	2795	3264
Spouse	0	0	0	0	708	1527	1012
Offspring	0	0	0	0	0	0	342
	6431	7918	8151	4753	6795	10,299	11,691

Note that it is possible for one person to have multiple 'roles', e.g. the fact that an individual is a twin does not exclude the possibility that this individual is also a spouse or a parent of a twin. For convenience, only one role is listed here for each person.

DATA COLLECTION IN THE NETHERLANDS TWIN REGISTRY

This thesis is based on data from the 6th and 7th surveys, sent to the adult participants of the Netherlands Twin registry in 2002 and 2004, respectively. Survey 6 (2002) was mailed to 29,217 individuals (14,162 twins and multiples, 3606 non-twin siblings and 11,449 parents of twins). Twins also received a survey for their spouses. The 2002 survey was completed and returned by a total of 10,299 individuals (Table 3.1). Survey 7 (2004) was sent to 28,859 individuals:

13,322 twins and multiples, 3420 siblings, 10,156 parents and 1961 partners. In total these individuals came from 7202 different families. Non-responders received a reminder in March 2005. The data collection was continued for several years, and by July 2009, the survey had been completed by a total of 11,691 individuals (Table 3.1). Survey 7 was also sent to the Dutch speaking families registered with the East Flanders Prospective Twin Survey in Belgium (Derom et al., 2006). By July 2009, 1800 Belgian participants had returned the survey. The data from the Belgian families were not included in this thesis.

At the time the datasets for this thesis were prepared, the data collection was still ongoing. For this reason, not all data could be included in the analyses. Chapter 8 includes the survey 7 data from 8645 individuals. Additional efforts were made in a later stage to collect questionnaire data in individuals who had not returned the questionnaire and whose questionnaire data were of particular importance, for instance because of their participation in the GAIN study, which involved the collection of DNA samples (Boomsma et al., 2008). These individuals received a new invitation and survey. As a result, additional questionnaire data of 254 participants could be included in Chapters 6 and 7, which are therefore based on a total sample size of 8899 for survey 7.

In total, this thesis includes data from a total of 10,299 participants for survey 6 and 8899 participants for survey 7. Of these individuals, 6794 participated in both surveys and 12,404 participated in at least one. After removal of individuals who did not answer the headache section (N = 84) and some individuals with unknown sex (N = 17) this resulted in a total number of 12,303 participants (5607 twins, 1772 siblings, 3280 parents and 1644 partners) who provided migraine data in at least one of the two surveys.

Migraine data

A detailed section about headache symptoms was included in surveys 6 (2002) and 7 (2004). Table 3.2 provides an overview of the headache items in each survey, and the number of respondents per item. The questions were identical in both surveys, although in survey 7 some minor additions were made. The questionnaire items can be translated to correspond with the International Headache Society (IHS) diagnostic criteria for migraine (Headache Classification Committee of the International Headache Society, 2004), as described in Table 3.3.

Two different methods are used to define affection status for migraine. The primary method is based on latent class analysis (LCA, see e.g. Lazarsfeld & Henry, 1968; McCutcheon, 1987), and is described in detail in Chapter 5. For reference to other studies, many results are also reported for the conventional IHS definition. This classification was made as follows: for a diagnosis of MO the individual was required to have symptom A, B, at least 2 of C2, C3 and C4, and at least 1 of D1 and D2. Individuals who met criteria for MO and also reported visual aura symptoms were classified as having MA. Note that an approximation of the IHS diagnostic criteria was used; an exact diagnosis was not possible due to the fact that no information on unilateral location of the headache was available. In addition, it was not possible to determine whether an individual had both photo- and phonophobia, or whether only one of the two was present. Therefore, the diagnosis made based on our data may differ slightly from the official IHS diagnosis of migraine.

Test-retest reliability of the migraine data

In July 2005, a subset of 240 participants of survey 7 who completed the survey in November 2004 (one per family, aged 30-40) were asked to complete a shortened version of the survey a second time, to investigate the reliability of the surveys. A total of 200 individuals returned the shortened version of the survey, and received a pedometer as a reward. 199 participants answered the headache section in both versions of the questionnaire. The tetrachoric test-retest correlation between the full and the shortened version of the questionnaire was .87 for the screening question ("Do you ever experience headache attacks, for instance migraine?"), and ranged between .82 (pulsation) and .92 (nausea/vomiting) for the IHS migraine symptoms (assuming the individuals who screened negative did not have the symptom). Similar correlations were observed between surveys 6 and 7: the correlation for the screening question was .87, and for the IHS migraine symptoms the correlations ranged between .82 (pulsation) and .87 (moderate to severe pain intensity), based on the data of the 6794 individuals who participated in both surveys.

Table 3.2

The headache items included in surveys 6 and 7 and the number of responses for each item			
Headache items		Availability (N)	
		Survey 6	Survey 7
1	Do you ever experience attacks of headache, for instance migraine? (no/yes)	10,183	8811
2	How often do you have these attacks of headache? 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 almost continuously*	2960	2635
3	How long do these headaches usually last? _____ hours and _____ minutes	2471	2095
4	The headache is usually (if multiple answers are applicable, only mention the most important one) a. pounding or stabbing b. pressing, as if a heavy weight is pressing on your head c. squeezing, as if there is a tight band around your head	2729	2474
5	How intense is the headache during most attacks? The intensity of the headache is usually: Mild Moderate Severe	2944	2569

Table 3.2 (continued)

6	During a headache attack, do you experience: (no/yes)			
	a.	aversion of light, sound or smell?	2875	2499
	b.	nausea or vomiting?	2846	2463
	c.	partial loss of vision, seeing flashes of light or (zigzag) patterns?	2839	2422
	d.	tingling sensations in arm or mouth, or speech disturbances?	2779	2365
	e.	muscle weakness causing difficulty walking or using your arm normally?*	0	2331
	f.	aggravation of headache by physical activity?	2857	2483
Total number of respondents in survey			**10,299**	**8899**

* Item included in survey 7 (2004) only.

Measures of depression

In the analyses of the comorbidity of migraine and depression (Chapters 6 and 7), several indicators of (anxious) depression were used. A measure available for the majority of the NTR sample (N = 9813) was an anxious depression factor score, calculated according to the approach developed in the Netherlands twin family study of anxious depression (NETSAD, Boomsma et al., 2000). This score was based on four variables measured in survey 6: the anxious depression scale from the Young Adult Self Report questionnaire (YASR, Achenbach, 1990), the somatic anxiety and neuroticism subscales of the Amsterdamse Biografische Vragenlijst (ABV, Wilde, 1970), and the Spielberger Trait-State Anxiety Inventory (STAI, Spielberger et al., 1970). A second measure used in Chapter 6 was the neuroticism scale of the NEO Five Factor Inventory (Costa & McCrae, 1992). This measure was available for 8567 individuals.

In addition, a subset of NTR participants underwent the Composite International Diagnostic Interview, version 2.1 (CIDI, Peters & Andrews, 1995; Wittchen, 1994), either as part of the NETSAD study or as part of the selection procedure for the GAIN study (Boomsma et al., 2008; Middeldorp et al., 2006). The CIDI is a standardized diagnostic interview designed to diagnose mental disorders, including major depressive disorder (MDD), based on the definitions of the ICD-10 (World Health Organization, 1993) and DSM-IV (American Psychiatric Association, 2001). Data from 112 individuals diagnosed with MDD based on a CIDI interview were included in Chapter 6.

TABLE 3.3

Correspondence between IHS migraine criteria and questionnaire items				
	IHS criteria	**Question***	**Unaffected**	**Affected**
A.	At least 5 attacks fulfilling criteria B-D	2	< several times a year	>= several times a year**
B.	Headache attacks lasting 4-72 hours (untreated or unsuccessfully treated)	3	< 4h or > 72h	between 4 and 72h
C.	Headache has at least two of the following characteristics:			
	1. unilateral location	-	n/a	n/a
	2. pulsating quality	4a	no	yes
	3. moderate or severe pain intensity	5	mild	moderate or severe
	4. aggravation by or causing avoidance of routine physical activity (e.g., walking or climbing stairs)	6f	no	yes
D.	During headache at least one of the following:			
	1. nausea and/or vomiting	6b	no	yes
	2. photophobia and phonophobia	6a	No	yes †
E.	Not attributed to another disorder	-	n/a	n/a
Aura	See Table 1.1	6c‡	no	yes

* Number refers to the questionnaire items listed in Table 3.2.

** Probably somewhat conservative, given the age of the sample.

† the criteria state that photo AND phonophobia should be present; however, the question in these surveys is not phrased such that this can be determined and might therefore give an overestimation of the number of individuals that satisfy D2.

‡ to maintain consistency over survey 6 and 7, only the visual aura question was used, although survey 7 also includes questions (items 6d and 6e in Table 3.2) intended to measure sensory aura and muscle weakness.

Genotyping

Linkage

A subset of NTR participants (3944 individuals from 841 nuclear families) was genotyped for linkage analysis. The DNA was extracted from either whole blood or buccal swabs, using standard protocols (Meulenbelt et al., 1995; Miller et al., 1988). The genotyping was done by the Mammalian Genotyping Service in Marshfield (400 microsattelites at 10 cM intervals) and the Molecular Epidemiology Section of the Leiden University Medical Centre (10 cM Applied Biosystems Human Linkage Set v2.5 MD10 with some additional markers; 419 microsattellites). These two screens were combined into one dataset. Headache data were available for 2536 of the genotyped individuals (1146 twins, including 188 MZ twin pairs, 858 parents and 532 siblings). The data of these participants were used in the linkage study described in Chapter 8.

Genome-wide association

Two subsets of NTR participants were genotyped at genome-wide SNP markers and included in the meta-analysis of genome-wide association (GWA) studies for migraine, described in Chapter 9.

The first NTR sample (N = 1863) was genotyped for the Genetic Association Information Network (GAIN) project. The genotyping was performed for a GWA study on MDD (Boomsma et al., 2008; Sullivan et al., 2008). This sample included 1703 non-depressed controls and 160 individuals with a lifetime diagnosis of DSM-IV MDD. After quality control, a total of 1593 individuals were left for whom genotypes and migraine data were available (112 with MDD, 1481 without MDD). DNA was extracted from whole blood samples and genotyping was performed by Perlegen Sciences, using a set of four proprietary, high-density oligonucleotide arrays (~600K SNPs). Genotypes were delivered for 599,156 SNPs, of which 435,291 (including 427,024 autosomal SNPs) survived quality control. Genotyping and quality control procedures are described in detail in Sullivan et al. (2008).

The second NTR sample was genotyped at 657,366 SNP markers, using the Human660W-Quad BeadChip. Quality control cut-offs were similar to those used with the first sample. SNPs were excluded based on MAF < 0.01, missing genotype rate > 0.05 or a p-value < 1×10^{-5} in a test of Hardy-Weinberg equilibrium. After quality control, 515,781 SNPs were left. Samples were excluded based on sample contamination (17 samples) or insufficient quality DNA (32 samples). A total of 1173 individuals were left after quality control;

migraine data were available for 1094 subjects. These data were included in the meta-analysis described in Chapter 9.

DATA COLLECTION IN THE NETHERLANDS STUDY OF DEPRESSION AND ANXIETY (NESDA)

Chapters 6 and 9 include headache data collected in the context of the Netherlands Study of Depression and Anxiety (NESDA, Penninx et al., 2008). The total number of NESDA participants was 2981. Of these participants, 2,601 provided headache data. Chapter 6 includes data of 1636 NESDA participants with MDD, who also provided headache data. Data from 1530 NESDA participants (1383 with MDD, 147 without MDD) were included in the meta-analysis of GWA studies, described in Chapter 9.

Measurement of migraine in NESDA

In the NESDA study, migraine was assessed with the same questionnaire items included in the 2002 survey of the NTR (Table 3.2). The items were included in a questionnaire that was part of a baseline assessment at the beginning of the NESDA study, to collect data on mood, lifestyle, medical history, and medication use (Licht et al., 2008).

Measurement of depression in NESDA

MDD was diagnosed based on version 2.1 of the Composite International Diagnostic Interview (CIDI, Peters & Andrews, 1995; Wittchen, 1994), a standardized diagnostic interview designed to diagnose mental disorders, based on the definitions of the ICD-10 (World Health Organization, 1993) and DSM-IV (American Psychiatric Association, 2001). Of the 2981 NESDA participants, 2601 completed a self-report questionnaire that provided information on migraine. Of these individuals, 1636 were diagnosed with lifetime MDD (1017 of whom had a diagnosis of MDD in the past year). The lifetime MDD variable was included in Chapter 6, in which migraine symptomatology is compared in lifetime and current MDD patients versus controls with a low risk of depression.

Genotyping

A subset of 1859 NESDA participants was genotyped according to the procedures described above for the NTR sample (Sullivan et al., 2008). After quality control, 1530 individuals were left for whom headache data were available. The data of these individuals were included in the meta-analysis of GWA studies for migraine (Chapter 9).

Other samples included in GWA meta-analysis

In addition to the NESDA sample and the two NTR samples, the meta-analysis of GWA studies (Chapter 9) included data from three European samples, the AGES-Reykjavik study (AGES-RS), the Erasmus Rucphen Family study (ERF) and the Rotterdam study.

AGES-RS

The AGES-Reykjavik study is a population-based cohort study established in 1967 to prospectively study cardiovascular disease in Iceland (Harris et al., 2007; Jonsdottir et al., 2002; Scher et al., 2009; Sigurdsson et al., 1995). Migraine data and genotypes were available for 3219 subjects, including 357 migraine cases and 2862 controls. Genotyping was performed on the Illumina 370CNV platform.

ERF

The Erasmus Rucphen Family study (ERF) is a family-based study in a genetically isolated population in the southwest of the Netherlands (Santos et al., 2006; Stam et al. 2010). The meta-analysis includes data from 1546 ERF participants; 330 migraineurs and 1216 controls. Genotyping was performed on several different platforms (Illumina HumanHap300, HumanHap370, Affymetrix 250K Nsp array).

Rotterdam Study

The Rotterdam study is a prospective population based cohort study among persons 55 years or older who were living in Ommoord, a well-defined district of Rotterdam, the Netherlands (Hofman et al., 2007). Migraine data were available for 1998 unrelated individuals from this study, including 349 cases and 1649 controls. Genotyping was performed using the Illumina Infinium II HumanHap550 chip.

References

Achenbach, T. M. (1990). The Young Adult Self Report. Burlington, VT: University of Vermont, Dept of Psychiatry.

American Psychiatric Association (2001). Diagnostic and Statistical Manual of Mental Disorders (4th ed.). Washington, DC: American Psychiatric Publishing.

Boomsma, D. I., Beem, A. L., van den Berg, M., Dolan, C. V., Koopmans, J. R., Vink, J. M., et al. (2000). Netherlands twin family study of anxious depression (NETSAD). Twin Res, 3(4), 323-334.

Boomsma, D. I., de Geus, E. J., Vink, J. M., Stubbe, J. H., Distel, M. A., Hottenga, J. J., et al. (2006). Netherlands Twin Register: from twins to twin families. Twin Res Hum Genet, 9(6), 849-857.

Boomsma, D. I., Vink, J. M., van Beijsterveldt, T. C., de Geus, E. J., Beem, A. L., Mulder, E. J., et al. (2002). Netherlands Twin Register: a focus on longitudinal research. Twin Res, 5(5), 401-406.

Boomsma, D. I., Willemsen, G., Sullivan, P. F., Heutink, P., Meijer, P., Sondervan, D., et al. (2008). Genome-wide association of major depression: description of samples for the GAIN Major Depressive Disorder Study: NTR and NESDA biobank projects. Eur J Hum Genet, 16(3), 335-342.

Costa, P. T., & McCrae, R. R. (1992). Revised NEO Personality Inventory (NEO-PI-R) and NEO Five-Factor Inventory (NEO-FFI); Professional Manual. Odessa, FL: Psychological Assessment Resources.

Derom, C. A., Vlietinck, R. F., Thiery, E. W., Leroy, F. O., Fryns, J. P., & Derom, R. M. (2006). The East Flanders Prospective Twin Survey (EFPTS). Twin Res Hum Genet, 9(6), 733-738.

Harris, T. B., Launer, L. J., Eiriksdottir, G., Kjartansson, O., Jonsson, P. V., Sigurdsson, G., et al. (2007). Age, Gene/Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. Am J Epidemiol, 165(9), 1076-1087.

Headache Classification Committee of the International Headache Society (2004). The International Classification of Headache Disorders: 2nd edition, Cephalalgia (Vol. 24 [Suppl 1], pp. 9-160).

Hofman, A., Breteler, M. M., van Duijn, C. M., Krestin, G. P., Pols, H. A., Stricker, B. H., et al. (2007). The Rotterdam Study: objectives and design update. Eur J Epidemiol, 22(11), 819-829.

Jonsdottir, L. S., Sigfusson, N., Gudnason, V., Sigvaldason, H., & Thorgeirsson, G. (2002). Do lipids, blood pressure, diabetes, and smoking confer equal risk of myocardial infarction in women as in men? The Reykjavik Study. J Cardiovasc Risk, 9(2), 67-76.

Lazarsfeld, P. F., & Henry, N. W. (1968). Latent structure analysis. New York: Houghton, Mifflin.

Licht, C. M., de Geus, E. J., Zitman, F. G., Hoogendijk, W. J., van Dyck, R., & Penninx, B. W. (2008). Association between major depressive disorder and heart rate variability in the Netherlands Study of Depression and Anxiety (NESDA). Arch Gen Psychiatry, 65(12), 1358-1367.

McCutcheon, A. L. (1987). Latent class analysis. Beverly Hills: Sage Publications.

Meulenbelt, I., Droog, S., Trommelen, G. J., Boomsma, D. I., & Slagboom, P. E. (1995). High-yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations. Am J Hum Genet, 57(5), 1252-1254.

Middeldorp, C. M., Cath, D. C., van den Berg, M., Beem, A. L., van Dyck, R., & Boomsma, D. I. (2006). The association of personality with anxious and depressive psychopathology. In T. Canli (Ed.), Biology of Personality and Individual Differences (pp. 251-272). New York: The Guilford Press.

Miller, S. A., Dykes, D. D., & Polesky, H. F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res, 16(3), 1215.

Penninx, B. W., Beekman, A. T., Smit, J. H., Zitman, F. G., Nolen, W. A., Spinhoven, P., et al. (2008). The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res, 17(3), 121-140.

Peters, L., & Andrews, G. (1995). Procedural validity of the computerized version of the Composite International Diagnostic Interview (CIDI-Auto) in the anxiety disorders. Psychol Med, 25(6), 1269-1280.

Santos, R. L., Zillikens, M. C., Rivadeneira, F. R., Pols, H. A., Oostra, B. A., van Duijn, C. M., et al. (2006). Heritability of fasting glucose levels in a young genetically isolated population. Diabetologia, 49(4), 667-672.

Scher, A. I., Gudmundsson, L. S., Sigurdsson, S., Ghambaryan, A., Aspelund, T., Eiriksdottir, G., et al. (2009). Migraine headache in middle age and late-life brain infarcts. Jama, 301(24), 2563-2570.Sigurdsson, E., Thorgeirsson, G., Sigvaldason, H., & Sigfusson, N. (1995). Unrecognized myocardial infarction: epidemiology, clinical characteristics, and the prognostic role of angina pectoris. The Reykjavik Study. Ann Intern Med, 122(2), 96-102.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). STAI Manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press.

Stam, A. H., de Vries, B., Janssens, A. C., Vanmolkot, K. R., Aulchenko, Y. S., Henneman, P., et al. (2010). Shared genetic factors in migraine and depression. Evidence from a genetic isolate. Neurology, 74(4), 288-294.

Sullivan, P. F., de Geus, E. J., Willemsen, G., James, M. R., Smit, J. H., Zandbelt, T., et al. (2008). Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. Mol Psychiatry.

Wilde, G. J. S. (1970). Neurotische labiliteit gemeten volgens de vragenlijstmethode (The questionnaire method as a means of measuring neurotic instability). Amsterdam: Van Rossen.

Wittchen, H. U. (1994). Reliability and validity studies of the WHO--Composite International Diagnostic Interview (CIDI): a critical review. J Psychiatr Res, 28(1), 57-84.

World Health Organization (1993). The ICD-10 Classification of Mental and Behavioural Disorders: Diagnostic Criteria for Research. Geneva.

4

Personality, health and lifestyle in a questionnaire family study: A comparison between highly cooperative and less cooperative families

Distel M.A., Ligthart L., Willemsen G., Nyholt D.R., Trull T.J., & Boomsma D.I. (2007). Personality, health and lifestyle in a questionnaire family study: a comparison between highly cooperative and less cooperative families. Twin Research and Human Genetics 10, 348-353.

Abstract

The effect of non-response on health and lifestyle measures has received extensive study, showing at most relatively modest effects. Non-response bias with respect to personality has been less thoroughly investigated. The present study uses data from responding individuals as a proxy for the missing data of their non-responding family members to examine the presence of non-response bias for personality traits and disorders as well as health and lifestyle traits. We looked at the Big Five personality traits, borderline personality disorder (BPD) features, attention-deficit/hyperactivity disorder, anger, and several measures of health (body mass index, migraine) and lifestyle (smoking, alcohol use). In general, outcomes tend to be slightly more favorable for individuals from highly cooperative families compared to individuals from less cooperative families. The only significant difference was found for BPD features ($p = .001$). However, the absolute difference in mean scores is very small, less than 1 point for a scale ranging from 0 to 72. In conclusion, survey data on personality, health and lifestyle are relatively unbiased with respect to non-response.

Introduction

If non-response influences data collected in survey research, this may seriously limit the validity of the findings. As such, non-response has received much attention and several methods have been used to estimate non-response bias in population studies. In some studies, respondents and non-respondents were compared with respect to information that was already available, using data from official population statistics registers or health insurance databases (Bergstrand et al., 1983; Etter & Perneger, 1997; Reijneveld & Stronks, 1999; van den Berg et al., 2006). In other studies, non-respondents were contacted by telephone or reply card to obtain information on the characteristics of interest. This information was used to estimate non-response bias (Hill et al., 1997; Korkeila et al., 2001; Vink et al., 2004). Longitudinal studies also provide information on differences between non-respondents and respondents. In some cases, non-respondents in a follow-up study can be characterized using information obtained at the beginning of the study (Eerola et al., 2005; Heath et al., 2001; van Loon et al., 2003). Vink and colleagues (2004) proposed an additional method to study non-response bias in family samples. When a trait has a familial component, a possible non-response bias can be estimated by using data from respondents as a proxy for the missing data of their non-responding family members. Data from highly cooperative families (i.e. many invited family members participate) are compared to data provided by the participating members of less cooperative families (i.e. few invited family members participate). A difference between these two groups indicates a possible non-response bias.

These various study designs tend to show that non-respondents smoke more often and drink more alcohol (Barchielli & Balzi, 2002; Heath et al., 2001; Hill et al., 1997; Kotaniemi et al., 2001; Macera et al., 1990; van Loon et al., 2003) . Also, non-respondents tend to be less educated, more often divorced or widowed, have lower annual incomes, and a lower socio-economic status (Barchielli & Balzi, 2002; Goyder, 2002; Korkeila et al., 2001). In most studies, no differences between respondents and non-respondents were found for body mass index (BMI), major depression and social anxiety (Eerola et al., 2005; Korkeila et al., 2001). Vink et al. (2004), however, found an effect for anxious depression. In conclusion, non-response has been found to influence a variety of traits, but in general the effects were small.

Non-response bias with respect to personality has been less extensively investigated than lifestyle variables such as smoking behaviour and alcohol use.

The few studies that examined the effect of non-response on personality focused on the Big Five personality traits. Dollinger and Leong (1993) investigated differences in personality between individuals who volunteered to be followed up in longitudinal research and individuals who did not. They found volunteers to be more agreeable, more open to experiences and a little more extraverted. Rogelberg et al. (2003) showed that respondents were more agreeable and more conscientious than non-respondents. These results suggest that non-response may be associated with personality as well as with lifestyle and other demographic factors. It is not unlikely that individuals with high scores on personality traits such as impulsivity, affective instability, relationship problems and identity problems, which are the core features of borderline personality disorder (BPD; American Psychiatric Association, 2001), are less likely to complete a survey. If this is true, non-respondents will exhibit more BPD features, resulting in an underrepresentation of individuals with BPD features in the study sample.

It is particularly important to quantify the effect of response bias in much needed population-based studies of personality and mental health. Most studies on personality and other mental health variables utilize clinical samples, but although clinical samples are very important, for example in characterizing the syndromes of a disorder and evaluating treatment programs, there are also some limitations. Clinical samples are always biased to some degree and not representative of the disorder as it appears in the community. In clinical settings, the most severe cases (the individuals seeking treatment) are more likely to be selected in a study sample. Thus while clinical studies tend to sample the most severe cases, non-response bias might cause affected individuals to be underrepresented in population studies.

In the present article we describe data from a Dutch family study on personality, health, and lifestyle and compare data on family members from highly cooperative and less cooperative families (Vink et al., 2004) to investigate to what extent non-response bias affects questionnaire data on personality.

METHODS

PARTICIPANTS

This study is part of an ongoing study on personality, health and lifestyle in twin families registered with the Netherlands Twin Register (NTR; Boomsma et al., 2006). Surveys on personality, health and lifestyle were sent to the twin families

every 2 to 3 years. For the present study data from the 2004 to 2005 survey were used. Twins and their siblings, parents and spouses were contacted by mail and invited to complete a survey which was enclosed with the letter. Questionnaires were sent to 27,666 individuals from 7036 families. The average number of family members in the families that were invited to complete a questionnaire was 3.9 (SD = 1.6).

Figure 4.1 shows an overview of the number of participants and the response rates in the study. The figure is subdivided into two groups; individuals who participated before (left side) and individuals who did not participate before (right side). Of those 16,612 individuals who participated at least once before in a study of the NTR, 7662 individuals (46.1%) returned the questionnaire. Of those who were sent the questionnaires, 11,054 had never before participated in NTR research, because they never returned a questionnaire or because they registered only recently and therefore were invited to complete a questionnaire for the first time. In this group 955 (8.6%) individuals completed the questionnaire. A group of 1378 individuals informed us after they received the invitation that they were not willing to participate for various reasons (e.g. death of co-twin, illness, lack of time, lack of interest). For the remaining non-respondents reasons for not participating are unknown. Part of the invited individuals did not actively register but were recruited in 1991 by contacting city councils in the Netherlands for the addresses of twins. It is therefore plausible that some of these individuals received the invitation but were unwilling to participate. Others, however, might not have received the invitation because they moved to a different address without informing the NTR. We therefore contacted a subgroup of each of the two groups of non-respondents for which the reason for non-response was unknown (those who participated at least once before [N = 8117] and those who never participated [N = 9554, see Figure 4.1]) by telephone and asked whether they received the questionnaire and what their reason was for not participating. Addresses were incorrect in 23.8% and 42.0% of the two groups, respectively. In other words, a substantial group of targeted participants never received the questionnaire. After adjusting for these estimated rates of incorrect addresses by subtracting the number of incorrect addresses from the number of sent questionnaires, the estimated 'true' response rates for the two groups were 52.2% and 13.6%, respectively.

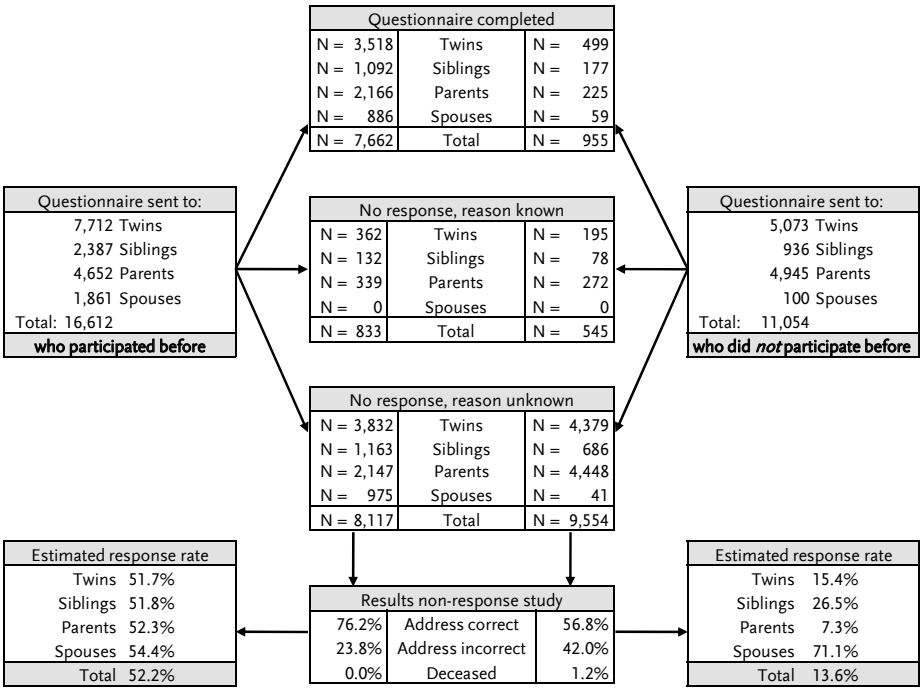


FIGURE 4.1

Overview of the number of participants in the study. The left side of the figure depicts the number of invited individuals who participated before and the right side depicts the number of invited individuals who did not participate before.

MEASURES

Personality-related traits

Borderline personality disorder features. BPD features were measured using the Personality Assessment Inventory-Borderline Features scale (PAI-BOR; Morey, 1991). The PAI-BOR consists of 24 items that are rated on a 4-point scale (0 to 3; *false, slightly true, mainly true, very true*). The items were scored according to Morey's test manual (Morey, 1991), which states that at least 80% of the items must have been completed to calculate a sum score and that missing and ambiguous answers should be substituted by a zero score. The English PAI-BOR was translated into Dutch and then translated back into English by a native

English speaking translator. This translation was reviewed and approved by the test author and publishing company (Psychological Assessment Resources). Because the data showed a somewhat right-skewed distribution, a square root data transformation was performed.

ADHD. The Conners' Adult ADHD Rating Scales (CAARS; Conners et al., 1999) was used to assess attention-deficit/hyperactivity disorder (ADHD). In this study, the subscales Inattentive and Hyperactive/Impulsive were used.

Big Five personality traits. The personality dimensions Neuroticism, Extraversion, Openness, Agreeableness and Conscientiousness were assessed using the NEO Five-Factor Inventory (NEO-FFI) which is the shortened version of the Revised NEO Personality Inventory (NEO-PI-R) developed by Costa and McCrae (1992).

Anger. Anger was measured using the Dutch adaptation of Spielberger's State-Trait Anger Scale (STAS; Spielberger et al., 1983; van der Ploeg et al., 1982). The trait version of the anger scale was administered, which measures how frequently an individual experiences state anger over time and in response to a variety of situations.

Health and lifestyle

Body Mass Index. BMI was calculated from self-reported height and weight by the formula: weight in kg / height in m^2.

Smoking. From the questions "Have you ever smoked?" *(no/a few times to try/yes)*, and "How often do you smoke at present?" *(I have quit smoking since .../once a week or less/several times a week but not daily/daily)* lifetime and current smoking status were determined. Lifetime smoking status was coded as 'smoked' *(yes)* versus 'never smoked' *(no/a few times to try)*. Current smoking status was coded as 'non-smoker' *(never smoked/a few times to try/quit smoking)* versus 'smoker' *(once a week or less/several times a week but not daily/daily)*.

Alcohol use. Regular alcohol use was determined by asking participants how often they used alcohol *(I don't drink alcohol/once a year or less/a few times a year/about once a month/a few times a month/once a week/several times a week/daily)*. *Several times a week* or more was treated as 'regular alcohol use'.

Also included in the survey were four items which together constitute the CAGE, a questionnaire designed to screen for possible alcohol problems (Ewing, 1984). Participants positive for two or more CAGE-items were classified as potentially having alcohol problems.

Migraine. Participants who screened positive for the question "Do you ever experience headache attacks, for instance migraine?" answered a series of follow-up questions concerning the characteristics of their headaches (frequency, duration, pulsating quality, pain intensity, aggravation by physical activity, and accompanying nausea and photo- or phonophobia). Based on this detailed symptom information a migraine diagnosis consistent with the International Headache Society criteria for migraine could be obtained (Headache Classification Committee of the International Headache Society, 2004).

Perceived health. Participants were asked to rate their general health on a 5-point scale *(poor, fair, reasonable, good, excellent)*. This variable was dichotomised to 'good' *(good, excellent)* and 'not good' *(poor, fair, reasonable)*.

DATA ANALYSES

Families in which at least one person completed the questionnaire were selected and categorized as highly cooperative families and less cooperative families, based on the percentage of invited family members that completed the questionnaire. When less than 80% of the invited family members completed the questionnaire, the family was considered a 'less cooperative family' and when 80% or more of the family members completed the questionnaire the family was considered a 'highly cooperative family'. The dataset contained 4499 participants from less cooperative families in which the mean percentage of participating individuals per family was 53% and 4118 participants from highly cooperative families in which the mean percentage of participating individuals per family was 94%. Multiple regression analyses (continuous measures) and logistic regression (categorical measures) were carried out in STATA 9.2 (StataCorp, College Station, Texas, USA) to determine the association between family cooperativeness and our selection of personality, health, and lifestyle variables, taking age and sex into account. Dummy coding was used for sex (0 = male, 1 = female) and family cooperativeness (0 = less cooperative, 1 = highly cooperative). Age was included in the analyses as a covariate. STATA's 'robust cluster' option was used to account for the non-independence of family

members. All other statistical analyses were performed in SPSS 13.0 for windows.

Since the traits of interest are not independent of each other PRELIS 2.45s (Jöreskog & Sörbom, 1996) was used to compute a correlation matrix of Pearson, polychoric and polyserial correlations for the 16 variables. We then estimated the equivalent number of measured independent traits using the matSpD interface (http://genepi.qimr.edu.au/general/daleN/matSpD/; Li & Ji, 2005; Nyholt, 2004). This analysis showed that the original 16 variables correspond to approximately 13 independent traits. To correct for multiple testing and to determine the significance of the results Bonferroni correction was applied by dividing the significance level by the number of independent traits. A p-value of 0.05/13 = 0.004 was considered significant.

Results

Mean values and prevalences of the various health, lifestyle and personality variables for individuals from highly and less cooperative families are shown in Table 4.1, as well as the results of the regression analyses. Individuals from highly cooperative families generally seem to have slightly more favorable outcomes than individuals from less cooperative families, but with the exception of BPD features, differences are not significant. Although BPD features are significantly more present in less cooperative families, the difference in BPD features between less cooperative and highly cooperative families is very small (0.76 point for males and 0.64 point for females), especially when considering the broad range of possible scores (0 - 72).

Table 4.1

Means (SD) and prevalences of personality, health and lifetime variables for males and females from less cooperative families and highly cooperative families

	Males		Females		Significance of cooperativeness*	
	L	H	L	H		
	N = 1,659	N = 1,675	N = 2,840	N = 2,443	F(df1, df2)	p
PAI-BOR	14.65 (±7.62)	13.89 (±7.29)	16.60 (±8.22)	15.96 (±8.03)	10.82 (1, 3264)	0.001
CAARS-Inattentive	6.07 (±3.51)	6.11 (±3.38)	6.09 (±3.39)	5.82 (±3.32)	2.86 (1, 3231)	0.091
CAARS-Hyperactive/impulsive	7.17 (±3.24)	7.01 (±3.22)	7.30 (±3.24)	7.02 (±3.11)	7.99 (1, 3231)	0.005
NEO-Neuroticism	27.97 (±6.77)	27.34 (±6.61)	31.01 (±7.37)	30.86 (±7.25)	3.46 (1, 3245)	0.063
NEO-Extraversion	41.12 (±5.89)	41.08 (±6.01)	41.40 (±5.98)	41.18 (±5.89)	0.24 (1, 3245)	0.624
NEO-Openness	36.53 (±5.88)	36.40 (±5.85)	37.14 (±5.62)	36.96 (±5.52)	0.70 (1, 3245)	0.404
NEO-Agreeableness	42.82 (±4.68)	42.89 (±4.72)	45.53 (±4.57)	45.61 (±4.45)	0.53 (1, 3245)	0.466
NEO-Conscientiousness	44.79 (±5.28)	44.97 (±5.24)	44.91 (±5.11)	45.30 (±5.03)	6.11 (1, 3245)	0.014
STAS-Anger	15.00 (±3.83)	14.83 (±3.79)	15.32 (±3.83)	15.13 (±3.74)	3.31 (1, 3266)	0.069
Body Mass Index	25.00 (±3.20)	24.90 (±3.22)	24.08 (±4.01)	23.95 (±3.92)	3.16 (1, 3253)	0.075

Table 4.1 (continued)

	Males		Females		Significance of cooperativeness*	
	L N = 1,659	H N = 1,675	L N = 2,840	H N = 2,443	F(df1, df2)	p
% lifetime smoking	58.3	54.8	47.0	45.0	6.92	0.009
% current smoking	25.7	22.3	20.0	18.9	3.24	0.072
% regular alcohol use	61.9	64.6	37.2	38.4	0.84	0.360
% potential alcohol problem	14.3	12.9	6.3	7.8	0.21	0.646
% migraine	4.5	3.8	13.6	14.2	0.04	0.846
% good to excellent health	84.8	87.6	84.8	84.8	3.35	0.067

L = individuals from less cooperative families, H = individuals from highly cooperative families. Means and standard deviations are presented for continuous variables and prevalences for categorical variables. Range of scales: PAI-BOR 0-72, CAARS 0-27, NEO 12-60, STAS 10-40.

*Comparisons are significant if $p < 0.004$ (Bonferroni correction) and corrected for age and sex.

DISCUSSION

In the present study, the response bias for several personality traits was investigated in a Dutch family sample. To examine whether non-response was trait-specific we also determined the response bias for several health and lifestyle measures. As expected, the participating members of less cooperative families showed somewhat higher scores on the PAI-BOR scale, suggesting non-response will be higher among subjects with more BPD features. However, the difference between people from less cooperative and highly cooperative families was relatively small, with a mean difference of less than 1 point (on a scale ranging from 0 to 72). This indicates that although the difference is statistically significant, its practical importance should not be overestimated. For some of the other measures, such as lifetime and current smoking, a similar trend was observed, with subjects from highly cooperative families having slightly more favorable outcomes, consistent with previous reports on smoking behavior. However, differences were very small; after correcting for multiple testing, none of these effects remained significant.

To examine whether our cut-off criterion of 80% family participation influenced our results we also examined 60%, 70% and 90% cut-off criteria. This did not significantly change the results.

Clearly, data from the relatives of non-respondents are only an approximation of the true values in the group of non-respondents; the outcomes of non-respondents may be less favorable than the outcomes of their participating relatives. However, considering the minor differences between participants from highly cooperative and less cooperative families, the true effect is not expected to be substantial. In conclusion, these results confirm previous findings that questionnaire data on personality, health and lifestyle are relatively unbiased with respect to non-response.

References

American Psychiatric Association (2001). Diagnostic and Statistical Manual of Mental Disorders (4th ed.). Washington, DC: American Psychiatric Publishing.

Barchielli, A., & Balzi, D. (2002). Nine-year follow-up of a survey on smoking habits in Florence (Italy): higher mortality among non-responders. Int J Epidemiol, 31(5), 1038-1042.

Bergstrand, R., Vedin, A., Wilhelmsson, C., & Wilhelmsen, L. (1983). Bias due to non-participation and heterogenous sub-groups in population surveys. J Chronic Dis, 36(10), 725-728.

Boomsma, D. I., de Geus, E. J., Vink, J. M., Stubbe, J. H., Distel, M. A., Hottenga, J. J., et al. (2006). Netherlands Twin Register: from twins to twin families. Twin Res Hum Genet, 9(6), 849-857.

Conners, C. K., Erhardt, D., & Sparrow, E. P. (1999). Conners' Adult ADHD Rating Scales (CAARS). Technical Manual. Toronto: Multi-Health Systems Inc.

Costa, P. T., & McCrae, R. R. (1992). Revised NEO Personality Inventory (NEO-PI-R) and NEO Five-Factor Inventory (NEO-FFI); Professional Manual. Odessa, FL: Psychological Assessment Resources.

Dollinger, S. J., & Leong, F. T. L. (1993). Volunteer bias and the 5-factor model. Journal of Psychology, 127, 29-36.

Eerola, M., Huurre, T., & Aro, H. (2005). The problem of attrition in a Finnish longitudinal survey on depression. Eur J Epidemiol, 20(1), 113-120.

Etter, J. F., & Perneger, T. V. (1997). Analysis of non-response bias in a mailed health survey. J Clin Epidemiol, 50(10), 1123-1128.

Ewing, J. A. (1984). Detecting alcoholism. The CAGE questionnaire. Jama, 252(14), 1905-1907.

Goyder, J. (2002). Evaluating socio-economic status (SES) bias in survey nonresponse. Journal of Official Statistics, 18, 1-11.

Headache Classification Committee of the International Headache Society (2004). The International Classification of Headache Disorders: 2nd edition, Cephalalgia (Vol. 24 [Suppl 1], pp. 9-160).

Heath, A. C., Howells, W., Kirk, K. M., Madden, P. A., Bucholz, K. K., Nelson, E. C., et al. (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.

Hill, A., Roberts, J., Ewings, P., & Gunnell, D. (1997). Non-response bias in a lifestyle survey. J Public Health Med, 19(2), 203-207.

Jöreskog, K., & Sörbom, D. (1996). PRELIS 2: User's reference guide. Chicago: Scientific Software International, Inc.

Korkeila, K., Suominen, S., Ahvenainen, J., Ojanlatva, A., Rautava, P., Helenius, H., et al. (2001). Non-response and related factors in a nation-wide health survey. Eur J Epidemiol, 17(11), 991-999.

Kotaniemi, J. T., Hassi, J., Kataja, M., Jonsson, E., Laitinen, L. A., Sovijarvi, A. R., et al. (2001). Does non-responder bias have a significant effect on the results in a postal questionnaire study? Eur J Epidemiol, 17(9), 809-817.

Li, J., & Ji, L. (2005). Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity, 95(3), 221-227.

Macera, C. A., Jackson, K. L., Davis, D. R., Kronenfeld, J. J., & Blair, S. N. (1990). Patterns of non-response to a mail survey. J Clin Epidemiol, 43(12), 1427-1430.

Morey, L. C. (1991). The Personality Assessment Inventory: Professional Manual. Odessa, FL: Psychological Assessment Resources.

Nyholt, D. R. (2004). A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet, 74(4), 765-769.

Reijneveld, S. A., & Stronks, K. (1999). The impact of response bias on estimates of health care utilization in a metropolitan area: the use of administrative data. Int J Epidemiol, 28(6), 1134-1140.

Rogelberg, S. G., Conway, J. M., Sederburg, M. E., Spitzmuller, C., Aziz, S., & Knight, W. E. (2003). Profiling active and passive nonrespondents to an organizational survey. J Appl Psychol, 88(6), 1104-1114.

Spielberger, C. D., Jacobs, G., Russell, S., & Crane, R. S. (1983). Assessment of anger: State Trait Anger Scale. In J. N. B. C. D. S. (Eds.) (Ed.), Advances in personality assessment (Vol. 2, pp. 159-187). Hillsdale, NJ: Erlbaum.

Van den Berg, G. J., Lindeboorn, M., & Dolton, P. (2006). Survey non-response and the duration of unemployment. Journal of the Royal Statistical Society Series A-Statistics in Society, 169, 585-604.

Van der Ploeg, H. M., Defares, P. B., & Spielberger, C. D. (1982). Handleiding bij de Zelf-analyse Vragenlijst. Lisse: Swets & Zeitlinger.

Van Loon, A. J. M., Tijhuis, M., Picavet, H. S. J., Surtees, P. G., & Ormel, J. (2003). Survey non-response in the Netherlands: Effects on prevalence estimates and associations. Annals of Epidemiology, 13, 105-110.

Vink, J. M., Willemsen, G., Stubbe, J. H., Middeldorp, C. M., Ligthart, R. S., Baas, K. D., et al. (2004). Estimating non-response bias in family studies: application to mental health and lifestyle. Eur J Epidemiol, 19(7), 623-630.

5

Migraine With Aura and Migraine Without Aura Are Not Distinct Entities: Further Evidence From a Large Dutch Population Study

Ligthart L., Boomsma D.I., Martin N.G., Stubbe J.H., & Nyholt D.R. (2006). Migraine with aura and migraine without aura are not distinct entities: further evidence form a large Dutch population study. Twin Research and Human Genetics, 9(1), 54-63.

ABSTRACT

It is often debated whether migraine with aura (MA) and migraine without aura (MO) are etiologically distinct disorders. A previous study using latent class analysis (LCA) in Australian twins showed no evidence for separate subtypes of MO and MA. The aim of the present study was to replicate these results in a population of Dutch twins and their parents, siblings and partners (N = 10,144). Latent class analysis of International Headache Society (IHS)-based migraine symptoms resulted in the identification of 4 classes: a class of unaffected subjects (class 0), a mild form of non-migrainous headache (class 1), a moderately severe type of migraine (class 2), typically without neurological symptoms or aura (8% reporting aura symptoms), and a severe type of migraine (class 3), typically with neurological symptoms, and aura symptoms in approximately half of the cases. Given the overlap of neurological symptoms and non-mutual exclusivity of aura symptoms, these results do not support the MO and MA subtypes as being etiologically distinct. The heritability in female twins of migraine based on LCA classification was estimated at .50 (95% confidence intervals [CI] .27–.59), similar to IHS-based migraine diagnosis (h^2 = .49, 95% CI .19–.57). However, using a dichotomous classification (affected–unaffected) decreased heritability for the IHS-based classification (h^2 = .33, 95% CI .00–.60), but not the LCA-based classification (h^2 = .51, 95% CI .23–.61). Importantly, use of the LCA-based classification increased the number of subjects classified as affected. The heritability of the screening question was similar to more detailed LCA and IHS classifications, suggesting that the screening procedure is an important determining factor in genetic studies of migraine.

Introduction

Migraine is a neurovascular disease characterized by a broad spectrum of symptoms, varying from headaches that are typically unilateral and have a pulsating quality, to various neurological symptoms such as nausea, increased sensitivity to light and sound (photophobia and phonophobia), and aura symptoms, which may consist of visual, sensory or motor disturbances (Headache Classification Committee of the International Headache Society, 2004).

A complicating factor in migraine research is the lack of clearly detectable biological markers that can help diagnose migraine. Therefore, diagnosis relies largely on symptomatology. The generally accepted diagnostic criteria for migraine are those published by the International Headache Society (Headache Classification Committee of the International Headache Society, 2004). These criteria were developed in order to standardize headache definitions and thereby facilitate comparisons between studies. The two main subtypes of migraine distinguished in these criteria are migraine without aura (MO) and migraine with aura (MA). However, it has been debated whether this distinction reflects true etiological differences between the disorders. Russell and Olesen (1995) found that first degree relatives of MA patients had a 3.8-fold risk of having MA, but no increased risk of having MO, suggesting distinct etiologies. In a study published in 1996, Russell et al. report different precipitating factors for MO and MA, and a low co-occurrence (4%) of the two disorders (Russell et al., 1996). However, other studies report higher co-occurrence of MO and MA. Launer et al. (1999), who conducted a large population-based study of migraine, found that 13% of patients had both MO and MA, which corresponds to 42% of all MA patients. Kallela et al. (2001) report co-occurrence of MO and MA in 41% of all migraineurs. However, these results may be biased due to the use of a clinical sample. Other evidence in support of MO and MA having shared etiologies is the fact that MO and MA are often found within the same family, and various types of migraine may be experienced by a single individual at different times in life (Ophoff et al., 1994). Thus, in spite of the traditional distinction between MO and MA, the frequent co-occurrence of the two types of attacks within families and within individuals suggests that a shared etiology may underlie MO and MA. Indeed, the recently updated International Headache Society (IHS) diagnostic criteria now include comments within the criteria for MA which state "Many patients who have frequent attacks with aura also have attacks without aura (code as 1.2 Migraine with aura and 1.1 Migraine without

aura)", and "The majority of migraine auras are associated with headache fulfilling criteria for 1.1 Migraine without aura".

Genetics of Migraine

Migraine has been shown to be under substantial genetic influence and is likely to be influenced by a large number of genes (Montagna, 2004). To date, three genes have been identified that are responsible for a rare autosomal dominant subtype of migraine with aura, called familial hemiplegic migraine (FHM). In 1996, various mutations were found in the calcium channel gene *CACNA1A*, located on chromosome 19p13, in five unrelated FHM families (Ophoff et al., 1996). In 2003, a second gene involved in FHM was identified on chromosome 1q23, the *ATP1A2* gene, which codes for the α2 subunit of the Na+/K+ pump (De Fusco et al., 2003). Dichgans et al. (2005) recently identified a third gene involved in FHM, located on chromosome 2q24. This gene, *SCN1A*, has previously been implicated in epilepsy. Some studies suggest that the *CACNA1A* gene may also play a role in the typical migraines (May et al., 1995; Nyholt et al., 1998; Terwindt et al., 2001), but negative findings have also been reported (Hovatta et al., 1994; Jones et al., 2001). Considering the clinical heterogeneity of migraine, and the fact that a variety of mutations in at least three different genes are implicated in a rare and specific subtype of migraine, it seems likely that many genes are involved in the pathogenesis of more common migraine types.

Through the years, various studies have investigated to what extent genes and environment influence migraine. The heritability of migraine is commonly estimated at 40 to 50% (Honkasalo et al., 1995; Larsson et al., 1995; Svensson et al., 2003). However, results have not always been consistent. Mulder et al. (2003) compared the prevalence and heritability of migraine in six different countries that participate in the GenomEUtwin project. Across countries, different questionnaires had been used to obtain data on migraine. The prevalence of migraine in females ranged from 10% in Finland to 34% in the Netherlands, and heritability estimates between 34% and 57% were found. In some countries evidence was found for non-additive genetic effects, but this was significant in Sweden only. This might be due to a lack of power to detect these effects, since very large samples are needed to detect non-additive genetic effects (Martin et al., 1978). A combined analysis of data from all countries suggested that non-additive effects might indeed play a role in migraine. However, demographic and ascertainment differences between countries might require to

first consider measurement issues and testing of measurement invariance (Lubke et al., 2004).

In most migraine studies, potentially affected subjects are identified with a screening question, for example, "Do you ever suffer from headache attacks, for instance migraine?" If participants answer this question with "yes", they will be asked further questions concerning more detailed features of their headaches, such as duration, frequency and specific symptoms. Consequently, differences in screening procedure (e.g., wording differences) have potential to significantly influence estimations of prevalence and heritability. Furthermore, cultural/translation (Guillemin et al., 1993), dietary (Millichap & Yee, 2003) and climate (Prince et al., 2004) differences may also influence these estimates.

Latent Class Analysis

In a previous study, Nyholt et al. (2004) used latent class analysis (LCA) to study migraine symptomatology in an Australian twin population. LCA was used to empirically identify subgroups of migraine patients in a population-based twin sample, and to examine whether these subtypes reflected distinct etiologies or different levels of severity on a single dimension. The results did not support an etiological distinction between MO and MA, but rather suggested a continuum underlying both migraine subtypes. The aim of the present article is to test the stability of the results from the Australian twin study by applying LCA to data from a sample of Dutch twins and their parents, siblings and partners. Furthermore, we aim to compare the use of LCA and IHS-based migraine classifications and to evaluate the influence of the screening question. Prevalence and heritability estimates are compared for several classifications of migraine, based on latent class analysis, IHS diagnostic criteria, and the screening question alone.

Methods

Sample

Data on migraine symptoms were collected in a large sample of Dutch twins, their parents, partners and siblings. The data were collected in 2002, as part of an ongoing family study on health, lifestyle and personality. The participants were volunteer members of the Netherlands Twin Registry, kept by the department of Biological Psychology at the Vrije Universiteit in Amsterdam. Questionnaires were mailed to 7261 families. The response rate for twins,

siblings and parents was approximately 30%. Sex was unknown for 10 subjects, who were consequently excluded from the analyses. Data from 10,144 participants were used; 4450 twins, 1446 siblings, 2743 parents, and 1505 partners. Of these 10,144 participants, 4239 (42%) were males and 5905 (58%) were females. The age of the participants ranged from 14.11 to 88.27 years, with a mean age of 41.4 years for males (SD = 14.7) and 38.9 for females (SD = 14.0). All subjects were included in the latent class analyses. Due to the small numbers of male twins screening positive for headache, genetic analyses were performed using only data from the female twins. Individuals for whom zygosity was unknown were excluded, resulting in a sample of 928 complete female twin pairs and 590 female twin individuals from incomplete pairs. For 25% of the pairs, DNA was used to determine zygosity. For the remaining pairs, zygosity was determined by means of questionnaire data on physical similarity and confusion of the twins by relatives, friends and strangers, resulting in a correct classification in approximately 97% of the cases. The mean age of the female twins was 33.3 years (SD = 11.5, range 17–85). The majority (85%) were between 20 and 50 years of age.

Participants who screened positive for the question "Do you ever experience headache attacks, for instance migraine?" answered a number of questions about the characteristics of their headache. These questions concerned the frequency and duration of the headaches, the quality of the headache (pounding, pressing or squeezing), and the severity. They were also asked whether any additional symptoms were present, such as sensitivity to light, sound or smell, nausea or vomiting, and aura symptoms, and whether the headache was aggravated by physical activity. This information was sufficient to obtain data on eight of the symptoms listed in the IHS criteria for migraine with and without aura, which allowed us to obtain migraine diagnoses consistent with IHS criteria (Tables 5.1A and 5.1B). Individuals satisfying IHS MO criteria also reporting visual aura symptoms were classified as having MA.

TABLE 5.1A

Diagnostic criteria for migraine without aura, as published by the International Headache Society (2004)
A. At least 5 attacks fulfilling criteria B-D
B. Headache attacks lasting 4-72 hours (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

TABLE 5.1B

LCA Symptom variables based on IHS criteria		
Code	**Abbreviation**	**Description**
A	>= 5 episodes	At least 5 episodes of migraine/headache during lifetime
B	4-72 hours	Headache attack usually lasts 4-72 hours
C2	Pulsating	The headache is usually pulsating
C3	Moderate or severe	The headache is usually moderate or severe
C4	Aggravation	Headache is aggravated by physical activity
D1	Nausea or vomiting	Headache is accompanied by nausea or vomiting
D2	Photophobia or phonophobia	Headache is accompanied by aversion of light or sound
Aura	Aura	Headache is accompanied by partial loss of vision, seeing flashes of light or zigzag patterns

Latent Class Analysis

Latent class analysis (e.g., Lazarsfeld & Henry, 1968; McCutcheon, 1987) has been described as a "categorical analog of factor analysis" (Kendler et al., 1996). A latent class cluster model describes the relationship between a set of observed variables and an unobserved, latent variable. The categories of this latent variable are called latent classes, or clusters. Given class membership, the observed variables are assumed to be independent. The parameters estimated in a latent class model are: (1) the prevalence of each class and (2) the probability, given class membership, that an individual will endorse a certain item. This results in a characteristic pattern of symptom endorsement for each of the classes. Each individual's most likely class membership is estimated based on his/her pattern of item endorsement. If the classes identified represent qualitatively different subtypes, we expect to find different patterns of symptom endorsement for different classes (i.e., symptom 1 might be more prevalent in class *x*, while symptom 2 might be more prevalent in class *y*). However, if there is one underlying continuous trait, classes will only differ by symptom severity (i.e., in class *y* all items are endorsed more frequently than in class *x*; Neuman et al., 1999). Because LCA is a model-based approach, it allows us to estimate the correct number of classes based on model fit and parsimony (e.g., Yeung et al., 2001). LCA can thus help us identify different classes of migraine patients within a sample, and give us an indication of whether these classes reflect separate migraine types with different etiologies, or merely different degrees of severity on the same dimension.

Latent class cluster models were tested using the Latent Gold 2.0 package (Statistical Innovations, Inc). The models utilized eight migraine symptom variables, each with three levels. For LCA of combined male and female data, sex was included as a covariate, to allow for differences in prevalence between males and females. Subjects who screened negative for the question: "Do you ever experience headache attacks, for instance migraine?" were assigned a value of 0 for each symptom; subjects who screened positive were assigned a value of 1 if they did not have the symptom, and a value of 2 if they did. Latent Gold allows users to include cases with missing data on dependent variables. Under this option, data are assumed to be missing at random (Vermunt & Magidson, 2000). When running Latent Gold, up to 10,000 iterations of the EM algorithm were allowed, and the estimation algorithm was restarted 500 times with different starting values to ensure global maximum likelihood estimates were obtained. The requested output included the classification details for each individual, the endorsement probabilities for each item within each class and

the bivariate residuals for each pair of variables, which indicate residual correlations between symptoms that are not explained by the latent class model. Model fits were compared using the Bayes Information Criterion (BIC; Schwarz, 1978), a measure of model fit that takes both sample size and model complexity into account. If the BIC of a more complex model fails to decrease, the simpler model (having the lower BIC) will be selected.

Genetic Analysis

The statistical program PRELIS 2.53 (Jöreskog & Sörbom, 1996) was used to test the fit of a multiple threshold model to the class membership data derived from the latent class analysis. A multiple threshold model assumes that the ordinal data are an imprecise measurement of an underlying normal distribution of liability (Neale & Cardon, 1992). The thresholds (expressed as z values) are the values that discriminate between categories. The area under the curve between thresholds thus represents the proportion of people in that category. Polychoric correlations for the twins were calculated using PRELIS, for each zygosity separately. A χ^2 goodness-of-fit test was used to assess the fit of the threshold model. A good fit of a multiple threshold model to the data would support the hypothesis that the categories reflect degrees of severity on a single dimension. Ninety-five per cent confidence intervals (CI) for the polychoric correlations were estimated in Mx 1.54 (Neale et al., 2003). Mx was also used for genetic model fitting. We first tested whether thresholds were equal in first- and second-born twins and in monozygotic (MZ) and dizygotic (DZ) twins. Using structural equation modeling, the variance of a trait can be decomposed into an additive genetic component (A), a shared environmental (C) or non-additive genetic (D) component, and a non-shared environmental component (E). Since the use of data from twins reared together does not allow us to estimate C and D simultaneously, separate ACE and ADE models were tested and compared. The significance of the variance components A, C and D was assessed by testing whether dropping them from the model resulted in a deterioration of fit. Model fit can be assessed using the −2 log likelihood (−2LL), which is χ^2 distributed. Nested models were compared using likelihood ratio tests (Δ−2LL), a significant increase in −2LL indicating a deterioration of model fit. Genetic models are also typically compared using the Akaike Information Criterion (AIC), a goodness-of-fit measure based on model fit and parsimony (AIC = −2LL minus two times the degrees of freedom). A lower AIC indicates a better model fit.

TABLE 5.2

Symptom prevalence and LCA classification by sex				
	Females (N = 5905)		Males (N = 4239)	
	N	%	N	%
Screening question	2178	36.9%	773	18.2%
>= 5 episodes (A1)	1921	32.5%	658	15.5%
4-72 hours (B)	1219	20.6%	374	8.8%
Pulsating (C2)	1129	19.1%	397	9.4%
Moderate / severe (C3a)	2002	33.9%	637	15.0%
Aggravation (C4)	1476	25.0%	435	10.3%
Nausea and/or vomiting (D1)	1045	17.7%	219	5.2%
Photo- and/or phonophobia (D2)	1414	23.9%	373	8.8%
Aura	707	12.0%	195	4.6%
Class 0	3730	63.2%	3469	81.8%
Class 1	120	2.0%	202	4.8%
Class 2	730	12.4%	333	7.9%
Class 3	1325	22.4%	235	5.5%

RESULTS

LATENT CLASS ANALYSIS

Of the total sample of 10,144 subjects, 2951 (29%) screened positive for the question: "Do you ever experience headache attacks, for instance migraine?" Seven hundred and seventy-three (26%) of these were males, and 2178 (74%) were females. Within the 2951 individuals screening positive, 2579 reported having headaches at least several times a year, 1593 participants had headaches lasting between 4 and 72 hours, and 1526 participants reported that their headache had a pulsating quality. Moderate or severe pain intensity was reported by 2639 individuals, and 1911 individuals reported aggravation of the headache by physical activity. Nausea or vomiting during a headache attack was reported by 1264 participants; photo- or phonophobia was reported by 1787 participants; and finally, 902 participants reported having visual aura symptoms during a headache attack (partial loss of vision, seeing flashes of light or zig-zag patterns). The prevalence of each symptom in males and females is listed in Table 5.2.

Latent class analysis was performed for the combined male and female data, followed by separate analyses for males and females. The latent classes identified were very similar across sex, suggesting that there were no qualitative sex differences in migraine symptoms (Figure 5.1). Three- and four-class models provided a similar fit to the data when parsimony was taken into account (producing BIC values of –608,726.55 and –608,725.83, respectively). However, unlike the three-class model, the four-class model produced no nominally significant ($p < .05$) bivariate residuals, thus indicating it provides a better explanation for the observed symptom correlations. For the four-class model, the combined analysis resulted in a more parsimonious fit (BIC = –608,726) than the separate analyses for males (BIC = –225,357) and females (BIC = –337,972), which sum to a comparatively larger BIC value of –563,329. Two- and five-class models provided a worse fit to the data, with substantially higher BIC values (–607,558 for a two-class model and –608,635 for a five-class model).

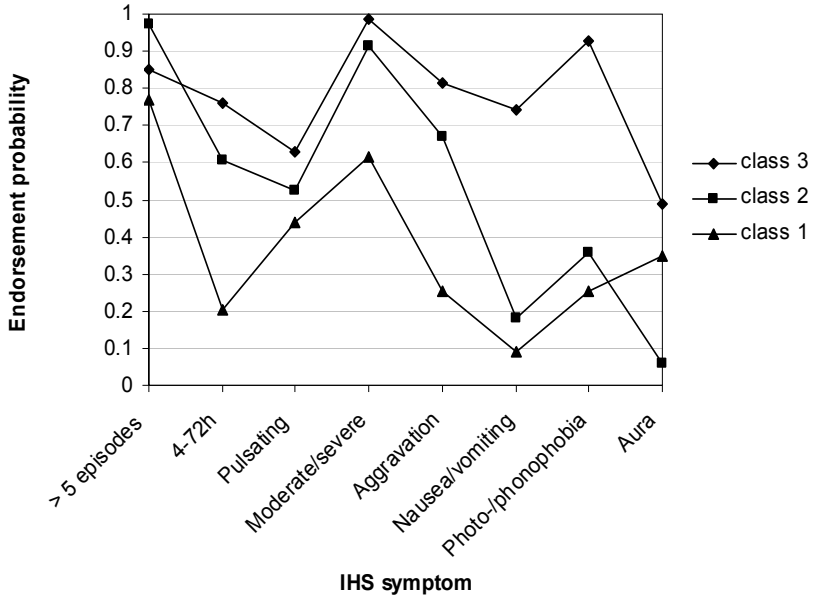


Figure 5.1

Symptom prevalence within each latent class. All endorsement probabilities in class 0 were zero (not shown).

The four classes derived from the most parsimonious model may be described as follows (Figure 5.1). Class 0 (not shown) describes subjects who screened negative and/or reported no migraine symptoms. Class 1 consists of subjects who have a mild form of non-migrainous headache, usually with moderate or severe pain intensity. These subjects typically do not have any of the other symptoms (i.e., the endorsement probabilities, which represent the proportion of individuals in each class presenting with each symptom, are less than 50%). Class 2 describes a moderately severe type of migrainous headache, typically without aura. It generally lasts 4 to 72 hours, is mostly pulsating, characterized by moderate or severe pain intensity, and aggravated by physical activity. Participants in class 2 usually do not have any of the neurological or aura symptoms (with endorsement probabilities of 18%, 34% and 6% for nausea/vomiting, photophobia/ phonophobia and aura, respectively). Finally, class 3 describes a severe type of migraine, which typically includes all IHS migraine symptoms. However, although the endorsement frequency for 'aura' is higher for class 3 than for any of the other classes, it is still only 49%. Interestingly, the endorsement frequency for 'aura' is higher in class 1 than in class 2, suggesting that there is a group of patients who have relatively mild headaches without neurological symptoms, but who experience visual aura symptoms.

Of the 5905 women, 3730 (63.2%) were estimated to be in class 0, 120 (2.0%) in class 1, 730 (12.4%) in class 2 and 1325 (22.4%) in class 3. Of the 4239 men, 3469 (81.8%) were estimated to be in class 0, 202 (4.8%) in class 1, 333 (7.9%) in class 2 and 235 (5.5%) in class 3 (Table 5.2). Although combining the data of males and females resulted in the most parsimonious fit and the LCA symptom profiles were similar across sex, separate analysis of males and females indicated that within the latent classes the prevalence of some migraine symptoms differed for males and females. After a correction for 32 comparisons, a number of symptoms showed significant ($p = .05$) sex differences within classes. In class 1 more males (24%, N = 52) than females (2%, N = 3) had attacks lasting 4 to 72 hours. Headache accompanied by nausea was more common in females (19%, N = 28) than in males (4%, N = 8), as was headache with photo- or phonophobia (females 39%, N = 59; males 16%, N = 34). Females also had a higher prevalence of visual aura symptoms (61%, N = 93) than men (25%, N = 55). In class 2, more males (47%, N = 148) than females (28 %, N = 205) had photo- or phonophobia during headache, and more females (8%, N = 60) than males (1%, N = 4) had visual aura symptoms. In class 3 more females (87%, N = 633) than males (74%, N = 235) had had at least five

episodes of headache or migraine, and headache lasting 4 to 72 hours was also more prevalent in females (78%, N = 570) than in males (64%, N = 204).

Heritability

Our next step was to perform genetic analyses on the LCA classification data for the twins. We obtained LCA classifications for 637 MZ and 291 DZ female twin pairs. Data for the males were available for only 236 MZ and 100 DZ pairs. As a result, we observed only two male–male DZ pairs where both twins screened positive. Consequently, we restricted our genetic analyses to the female population.

Polychoric twin correlations and 95% confidence intervals (95% CI) for the LCA classification are shown in Table 5.3. A χ^2 goodness-of-fit test for a multiple threshold model was performed on the twin correlations for the MZ and DZ twins separately (data not shown). None of these tests reached the nominal significance level of 5%, indicating that a multiple threshold model provides a good fit to the data. Thresholds were equal across zygosity and for first- and second-born twins. In the best fitting model the thresholds were estimated at .32, .44 and .85.

Results of testing an ACE model on the LCA four-class scheme indicated substantial influence of genetic factors, but no evidence for shared environmental influences. In an AE model, additive genetic factors explained 50% (95% CI = 41–59) of the variance. In an ADE model, the contribution of additive genetic effects was estimated at 25%, while non-additive genetic effects explained 27% of the variance (Table 5.3). Although the 95% CI for both the A and D components included zero, dropping both of them from the model resulted in a significant deterioration in fit (Δ–2LL = 95.441, 2df, $p < .001$).

The four-group IHS classification produced very similar polychoric twin correlations to the four-group LCA classification, resulting in similar overall heritabilities under the ACE model of .49 (95% CI = .19–.57) and 0.50 (95% CI = .27–.59), respectively. This indicates that use of the LCA classification does not lead to a loss of genetic information, compared to the IHS classification. Interestingly, the contribution of non-additive effects was substantially lower for the IHS classification.

Table 5.3

Polychoric correlations (r) and variance components for 4- and 2-group LCA and IHS classifications, and for screening question								
Classification	**r**	**95% CI**	**A (95% CI)**	**C (95% CI)**	**E (95% CI)**	**A (95% CI)**	**D (95% CI)**	**E (95% CI)**
LCA 4-group								
MZ	0.52	(.42-.60)	0.50	0.00	0.50	0.25	0.27	0.48
DZ	0.19	(.03-.34)	(.27-.59)	(.00-.20)	(.41-.59)	(.00-.58)	(.00-.60)	(.40-.58)
IHS 4-group								
MZ	0.49	(.40-.57)	0.49	0.00	0.51	0.43	0.06	0.51
DZ	0.23	(.08-.37)	(.19-.57)	(.00-.27)	(.43-.60)	(.00-.60)	(.00-.56)	(.43-.59)
LCA 2-group								
MZ	0.53	(.42-.62)	0.51	0.00	0.49	0.22	0.31	0.47
DZ	0.19	(.00-.37)	(.23-.61)	(.00-.25)	(.39-.59)	(.00-.60)	(.00-.62)	(.38-.58)
IHS 2-group								
MZ	0.46	(.29-.60)	0.33	0.13	0.54	0.47	0.00	0.53
DZ	0.29	(.04-.52)	(.00-.60)	(.00-.51)	(.40-.71)	(.00-.60)	(.00-.58)	(.40-.69)
Screening Question								
MZ	0.53	(.42-.62)	0.52	0.00	0.48	0.28	0.25	0.47
DZ	0.20	(.02-.38)	(.22-.61)	(.00-.26)	(.39-.58)	(.00-.61)	(.00-.62)	(.38-.58)

Full ACE and ADE models and confidence intervals are shown. A = additive genetic factors; C = shared environmental factors; D = non-additive genetic factors; E = non-shared environmental factors; SQ = screening question.

The four-group LCA and IHS classifications were then compared to clinically relevant two-group classifications (affected vs. unaffected). Table 5.3 shows results for the two-group LCA classification (treating class 0 and 1 as unaffected and class 2 and 3 as affected) and the two-group IHS classification (migraine vs. no migraine). The two-group LCA classification produces results very similar to the four-class scheme, whereas use of the two-group IHS classification resulted in a decrease in both the magnitude and precision (as reflected in the wider confidence intervals) of the heritability estimates compared to the four-group IHS scheme. This suggests a poorer correspondence between genetic risk and IHS groupings compared to the LCA groupings. Furthermore, as can be seen in Table 5.4, a substantial number (62%) of the individuals classified as LCA class 2 or 3, do not satisfy the criteria for IHS migraine.

Finally, we analyzed the heritability of the screening question alone. This two-group classification produced polychoric correlations and heritability estimates very similar to those of the other classifications, suggesting that the screening question is a very important determining factor for the genetic analyses performed on the more detailed symptom data and subsequent endpoint diagnoses.

Table 5.4

Crosstabulation of LCA and IHS diagnoses for female twins (N = 2446)				
	IHS SQ-	IHS SQ+	IHS SQ+, MO	IHS SQ+, MA
LCA Class 0 (SQ-)	1530	0	0	0
LCA Class 1	0	108	0	0
LCA Class 2	0	279	45	0
LCA Class 3	0	222	140	122

SQ+ = screening positive, SQ- = screening negative, MO = migraine without aura, based on IHS criteria, MA = migraine with aura, based on IHS criteria.

Table 5.5

Comparison of Dutch and Australian twin samples: number of individuals positive for screening question, symptom or endpoint diagnosis, percentage of total, and percentage of those screening positive

	Dutch sample (N = 4448*)						Australian sample (N = 6212)					
	Females			Males			Females			Males		
	N	% of N	% of Ns	N	% of N	% of Ns	N	% of N	% of Ns	N	% of N	% of Ns
Total (N)	3031			1417			3438			2774		
Screening question (Ns)	1124	37.1%	-	270	19.1%	-	1777	51.7%	-	888	32.0%	-
A1 >= 5 episodes	996	32.9%	88.6%	232	16.4%	85.9%	1294	37.6%	72.8%	689	24.8%	77.6%
B 4-72h	607	20.0%	54.0%	128	9.0%	47.4%	1053	30.6%	59.3%	444	16.0%	50.0%
C2 Pulsating	579	19.1%	51.5%	151	10.7%	55.9%	1465	42.6%	82.4%	708	25.5%	79.7%
C3a Moderate / severe	1026	33.9%	91.3%	226	15.9%	83.7%	1576	45.8%	88.7%	708	25.5%	79.7%
C4 Aggravation	776	25.6%	69.0%	164	11.6%	60.7%	-	-	-	-	-	-
D1 Nausea and/or vomiting	507	16.7%	45.1%	79	5.6%	29.3%	980	28.5%	55.1%	344	12.4%	38.7%
D2 Photo- and/or phonophobia	718	23.7%	63.9%	120	8.5%	44.4%	1013	29.5%	57.0%	356	12.8%	40.1%
Aura	359	11.8%	31.9%	53	3.7%	19.6%	924	26.9%	52.0%	394	14.2%	44.4%

Table 5.5 (continued)

	Dutch sample (N = 4448*)						Australian sample (N = 6212)					
	Females			Males			Females			Males		
	N	% of N	% of Ns	N	% of N	% of Ns	N	% of N	% of Ns	N	% of N	% of Ns
MO	380	12.5%	33.8%	53	3.7%	19.6%	703	20.4%	39.6%	250	9.0%	28.2%
MA †	146	4.8%	13.0%	15	1.1%	5.6%	432	12.6%	24.3%	166	6.0%	18.7%
IHS-migraine	380	12.5%	33.8%	53	3.7%	19.6%	703	20.4%	39.6%	250	9.0%	28.2%
CL2	397	13.1%	35.3%	127	9.0%	47.0%	781	22.7%	44.0%	486	17.5%	54.7%
CL3	600	19.8%	53.4%	69	4.9%	25.6%	793	23.1%	44.6%	198	7.1%	22.3%
LCA-migraine	997	32.9%	88.7%	196	13.8%	72.6%	1574	45.8%	88.6%	684	24.7%	77.0%

Ns = number of individuals screening positive.

*Does not include the twins' parents, siblings and partners.

† MA individuals also fulfill criteria for MO.

DISCUSSION

Analogous to the results of Nyholt et al. (2004) utilizing Australian migraine data, latent class analysis of Dutch migraine data suggests the existence of four classes based on IHS migraine criteria: a subgroup of individuals who screened negative for the question "Do you ever experience headache attacks, for instance migraine?" and/or reported no IHS symptoms (class 0), a subgroup of participants who had a mild form of non-migrainous headache (class 1), a subgroup with a moderately severe type of migrainous headache, typically without neurological symptoms or aura (class 2), and a subgroup with a severe type of migraine, typically including all IHS migraine symptoms, and in approximately 50% of the cases, aura symptoms. These results do not support the MO and MA subtypes as being etiologically distinct. Although the frequency of aura is very low in class 2 and highest in class 3, more than 50% of patients in class 3 do not report aura symptoms. Our data suggest that it is the severity, number and combination of symptoms (in particular the presence of neurological symptoms) that distinguishes between classes, rather than the simple presence of aura symptoms.

The heritability of four-class LCA migraine in female twins was estimated at 50%. Non-shared environment explained the remaining 50% of variance, and no evidence was found for shared environmental influences. This estimate remained relatively stable across a variety of classifications, utilizing both LCA and IHS-based diagnosis. However, using a two-group IHS classification (migraine vs. no migraine) resulted in a decrease of the heritability estimate (33%), suggesting a poorer correspondence between genetic risk and IHS groupings, compared to LCA groupings. A similar decrease in heritability was observed by Nyholt et al. (2004).

Overall, results are similar for the Dutch and Australian populations. However, there are some interesting differences. Table 5.5 lists the positive screening rates, and the prevalence of individual symptoms and endpoint diagnoses for the Dutch and Australian twin samples. In the Dutch study, individuals were screened for potential migraine using the question: "Do you ever experience headache attacks, for instance migraine?" This resulted in 37% of females and 19% of males screening positive. The screening question used in the Australian study was "Have you ever had migraine or recurrent attacks of headaches?", resulting in 52% of females and 32% of males screening positive. The number of participants diagnosed as having LCA or IHS migraine is also

lower in the Dutch population, probably as a consequence of the lower number of participants screening positive.

Interestingly, a relatively low prevalence of aura and pulsating headache was found in the Dutch population, whereas the number of individuals reporting at least five headache episodes was relatively high. These discrepancies may possibly be explained by differences in ascertainment.

In the Australian study, aura symptoms were described as "visual problems such as blurring, showers of light, blind spots or double vision", whereas in the Dutch questionnaire they were described as "partial loss of vision, seeing flashes of light or (zigzag) patterns". This difference in the definition of visual aura symptoms might be responsible for the lower reported prevalence of visual aura in the Dutch population.

Furthermore, in the Australian study the participants were asked how many attacks of headache they had had in their lifetime. In the Dutch study, participants were asked how often their attacks occurred (i.e., the number of attacks per week/month/year). Individuals who had attacks at least several times a year were assumed to fulfill the criterion of having had at least five attacks in a lifetime. However, since this would be expected to be a conservative cut-off, the high prevalence resulting from this procedure is unexpected.

The question concerning pulsating headache was phrased similarly in both studies. The Dutch participants were asked if their headache was usually "throbbing or stabbing", whereas the Australian participants were asked if their headaches were usually experienced as "throbbing, pulsating or pounding - like being stabbed with a sharp knife". A possible explanation for the lower prevalence of this symptom in the Dutch population is that in the Dutch study a questionnaire was used, whereas in the Australian study, data were obtained through a telephone interview. Indeed, we expect data collected via telephone interview to be more accurate, as it allows subjects to ask the interviewer for a clarification of a question or description. Thus, this difference in data collection could also explain other prevalence differences between the two studies.

Finally, for the Dutch cohort, no data were collected on whether headache was unilateral or whether it prohibited daily activities. The Australian study, on the other hand, did not include data on aggravation of headache by physical activity, whereas the present study did. However, considering the high correlations between the reporting of these individual migraine symptoms (Nyholt et al., 2004), these differences are unlikely to significantly alter the LCA results.

Despite differences in the data collection procedures used, the populations examined, and the age range of the subjects, both latent class and genetic analyses yielded similar results for the present (Dutch) and Australian study. Our findings support earlier evidence that migraine is influenced by genetic factors (with some indication for non-additive effects) and non-shared environment, but not by shared environment. In addition, our LCA results further support the hypothesis that MO and MA are not etiologically distinct disorders.

Furthermore, our results indicate that in questionnaire- based migraine research, it is of vital importance to use an appropriate and sensitive screening question. The heritability of the screening question was very similar to the heritability of two-group LCA- and IHS-migraine, suggesting that the screening question is an important determining factor for the results of genetic analyses performed on the more detailed symptom data and endpoint diagnoses. A closer look at the contingency tables for the screening question, two-group LCA-migraine and two-group IHS-migraine (Table 5.6) shows that the large number of concordant unaffected pairs is likely to significantly influence the tetrachoric/ polychoric correlations on which the genetic analyses are based.

TABLE 5.6

Concordance rates for 2-group LCA-migraine, 2-group IHS-migraine and the screening question in female twins

	Screening Question					IHS-migraine					LCA-migraine			
	MZ		DZ			MZ		DZ			MZ		DZ	
	-	+	-	+		-	+	-	+		-	+	-	+
-	308	90	120	61	-	505	50	223	29	-	339	87	136	60
+	103	136	59	51	+	56	26	29	10	+	97	114	55	40

+ = affected, - = unaffected, MZ = monozygotic twin pairs, DZ = dizygotic twin pairs

A related issue is the influence of the screening question on findings regarding migraine prevalence. The different prevalence found in the Dutch and Australian populations (Table 5.5) may in part reflect real differences in migraine prevalence between Australia and the Netherlands, caused by cultural, environmental or genetic factors. For example, one would expect MA individuals would similarly answer yes to either the Australian and Dutch screening questions. However, even within the Dutch population large differences in

positive screening rate are found between two questionnaires that used different screening questions. An earlier questionnaire-based Dutch twin study, conducted in 1991, used the screening question: "Do you ever suffer from headaches?" This resulted in a positive screening rate of 66%, whereas in the present study (which used the question, "Do you ever experience headache attacks, for instance migraine?") only 29% screened positive. Using a screening question that excludes too many potential migrainous headache sufferers will lead to unnecessary loss of valuable symptom data and bring into question the validity of an unaffected status.

Finally, analogous to the results of Nyholt et al. (2004), the LCA-based approach resulted in a larger number of migrainous headache patients being classified, and a higher heritability, compared to the IHS-based approach. This suggests that the use of an LCA-based approach has the potential to increase power in genetic studies of migraine. Indeed, two recent genome-wide linkage scans (Lea et al., 2005; Nyholt et al., 2005) found significantly increased evidence for linkage utilizing an LCA-based migrainous headache definition compared to migraine diagnosed according to strict IHS criteria.

REFERENCES

De Fusco, M., Marconi, R., Silvestri, L., Atorino, L., Rampoldi, L., Morgante, L., et al. (2003). Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. Nat Genet, 33(2), 192-196.

Dichgans, M., Freilinger, T., Eckstein, G., Babini, E., Lorenz-Depiereux, B., Biskup, S., et al. (2005). Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. Lancet, 366(9483), 371-377.

Guillemin, F., Bombardier, C., & Beaton, D. (1993). Cross-cultural adaptation of health-related quality of life measures: literature review and proposed guidelines. J Clin Epidemiol, 46(12), 1417-1432.

Headache Classification Committee of the International Headache Society (2004). The International Classification of Headache Disorders: 2nd edition, Cephalalgia (Vol. 24 [Suppl 1], pp. 9-160).

Honkasalo, M. L., Kaprio, J., Winter, T., Heikkila, K., Sillanpaa, M., & Koskenvuo, M. (1995). Migraine and concomitant symptoms among 8167 adult twin pairs. Headache, 35(2), 70-78.

Hovatta, I., Kallela, M., Farkkila, M., & Peltonen, L. (1994). Familial migraine: exclusion of the susceptibility gene from the reported locus of familial hemiplegic migraine on 19p. Genomics, 23(3), 707-709.

Jones, K. W., Ehm, M. G., Pericak-Vance, M. A., Haines, J. L., Boyd, P. R., & Peroutka, S. J. (2001). Migraine with aura susceptibility locus on chromosome 19p13 is distinct from the familial hemiplegic migraine locus. Genomics, 78(3), 150-154.

Jöreskog, K., & Sörbom, D. (1996). PRELIS 2: User's reference guide. Chicago: Scientific Software International, Inc.

Kallela, M., Wessman, M., Havanka, H., Palotie, A., & Farkkila, M. (2001). Familial migraine with and without aura: clinical characteristics and co-occurrence. Eur J Neurol, 8(5), 441-449.

Kendler, K. S., Eaves, L. J., Walters, E. E., Neale, M. C., Heath, A. C., & Kessler, R. C. (1996). The identification and validation of distinct depressive syndromes in a population-based sample of female twins. Arch Gen Psychiatry, 53(5), 391-399.

Larsson, B., Bille, B., & Pedersen, N. L. (1995). Genetic influence in headaches: a Swedish twin study. Headache, 35(9), 513-519.

Launer, L. J., Terwindt, G. M., & Ferrari, M. D. (1999). The prevalence and characteristics of migraine in a population-based cohort: the GEM study. Neurology, 53(3), 537-542.

Lazarsfeld, P. F., & Henry, N. W. (1968). Latent structure analysis. New York: Houghton, Mifflin.

Lea, R. A., Nyholt, D. R., Curtain, R. P., Ovcaric, M., Sciascia, R., Bellis, C., et al. (2005). A genome-wide scan provides evidence for loci influencing a severe heritable form of common migraine. Neurogenetics, 6(2), 67-72.

Lubke, G. H., Dolan, C. V., & Neale, M. C. (2004). Implications of absence of measurement invariance for detecting sex limitation and genotype by environment interaction. Twin Res, 7(3), 292-298.

Martin, N. G., Eaves, L. J., Kearsey, M. J., & Davies, P. (1978). The power of the classical twin study. Heredity, 40(1), 97-116.

May, A., Ophoff, R. A., Terwindt, G. M., Urban, C., van Eijk, R., Haan, J., et al. (1995). Familial hemiplegic migraine locus on 19p13 is involved in the common forms of migraine with and without aura. Hum Genet, 96(5), 604-608.

McCutcheon, A. L. (1987). Latent class analysis. Beverly Hills: Sage Publications.

Millichap, J. G., & Yee, M. M. (2003). The diet factor in pediatric and adolescent migraine. Pediatr Neurol, 28(1), 9-15.

Montagna, P. (2004). The physiopathology of migraine: the contribution of genetics. Neurol Sci, 25 Suppl 3, S93-96.

Mulder, E. J., Van Baal, C., Gaist, D., Kallela, M., Kaprio, J., Svensson, D. A., et al. (2003). Genetic and environmental influences on migraine: a twin study across six countries. Twin Res, 6(5), 422-431.

Neale, M. C., Boker, S. M., Xie, G., & Maes, H. H. (2003). Mx: Statistical Modeling (6 ed.). VCU Box 900126, Richmond, VA 23298: Department of psychiatry.

Neale, M. C., & Cardon, L. R. (1992). Methodology for genetic studies in twins and families. Dordrecht: Kluwer Academic.

Neuman, R. J., Todd, R. D., Heath, A. C., Reich, W., Hudziak, J. J., Bucholz, K. K., et al. (1999). Evaluation of ADHD typology in three contrasting samples: a latent class approach. J Am Acad Child Adolesc Psychiatry, 38(1), 25-33.

Nyholt, D. R., Gillespie, N. G., Heath, A. C., Merikangas, K. R., Duffy, D. L., & Martin, N. G. (2004). Latent class and genetic analysis does not support migraine with aura and migraine without aura as separate entities. Genet Epidemiol, 26(3), 231-244.

Nyholt, D. R., Lea, R. A., Goadsby, P. J., Brimage, P. J., & Griffiths, L. R. (1998). Familial typical migraine: linkage to chromosome 19p13 and evidence for genetic heterogeneity. Neurology, 50(5), 1428-1432.

Nyholt, D. R., Morley, K. I., Ferreira, M. A., Medland, S. E., Boomsma, D. I., Heath, A. C., et al. (2005). Genomewide significant linkage to migrainous headache on chromosome 5q21. Am J Hum Genet, 77(3), 500-512.

Ophoff, R. A., Terwindt, G. M., Vergouwe, M. N., van Eijk, R., Oefner, P. J., Hoffman, S. M., et al. (1996). Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell, 87(3), 543-552.

Ophoff, R. A., van Eijk, R., Sandkuijl, L. A., Terwindt, G. M., Grubben, C. P., Haan, J., et al. (1994). Genetic heterogeneity of familial hemiplegic migraine. Genomics, 22(1), 21-26.

Prince, P. B., Rapoport, A. M., Sheftell, F. D., Tepper, S. J., & Bigal, M. E. (2004). The effect of weather on headache. Headache, 44(6), 596-602.

Russell, M. B., & Olesen, J. (1995). Increased familial risk and evidence of genetic factor in migraine. Br Med J, 311(7004), 541-544.

Russell, M. B., Rasmussen, B. K., Fenger, K., & Olesen, J. (1996). Migraine without aura and migraine with aura are distinct clinical entities: a study of four hundred and eighty-four male and female migraineurs from the general population. Cephalalgia, 16(4), 239-245.

Schwarz, G. (1978). Estimating the dimension of a model. The Annals of Statistics, 6, 461-464.

Svensson, D. A., Larsson, B., Waldenlind, E., & Pedersen, N. L. (2003). Shared rearing environment in migraine: results from twins reared apart and twins reared together. Headache, 43(3), 235-244.

Terwindt, G. M., Ophoff, R. A., van Eijk, R., Vergouwe, M. N., Haan, J., Frants, R. R., et al. (2001). Involvement of the CACNA1A gene containing region on 19p13 in migraine with and without aura. Neurology, 56(8), 1028-1032.

Vermunt, J. K., & Magidson, J. (2000). Latent Gold User's Guide. Belmont: Statistical Innovations, Inc.

Yeung, K. Y., Fraley, C., Murua, A., Raftery, A. E., & Ruzzo, W. L. (2001). Model-based clustering and data transformations for gene expression data. Bioinformatics, 17(10), 977-987.

6

Migraine Symptomatology and Major Depressive Disorder

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ABSTRACT

Migraine and major depressive disorder (MDD) frequently co-occur, but it is unsure whether depression is associated with a specific subtype of migraine. The objective of this study was to investigate whether migraine is qualitatively different in MDD patients (N = 1816) and non-depressed controls (N = 3428). Migraine symptom data were analyzed using multi-group Latent Class Analysis, and a qualitative comparison was made between the symptom profiles of MDD patients and controls, while allowing for differences in migraine prevalence and severity between groups. In both groups, three migrainous headache classes were identified, which differed primarily in terms of severity. Both mild and severe migrainous headaches were two to three times more prevalent in MDD patients. Migraine symptom profiles showed only minor qualitative differences in the MDD and non-MDD groups: in the severe migrainous headache class, significant differences were observed only in the prevalence of aggravation by physical activity (83% and 91% for the non-MDD and MDD groups, respectively) and aura (42% vs. 53%, respectively). The similar overall symptom profiles observed in the MDD and non-MDD subjects suggest that a similar disease process may underlie migraine in both groups.

Introduction

A vast amount of literature describes the comorbidity of migraine and major depressive disorder (MDD). Comorbidity studies of depression and the two most common types of migraine—migraine with aura (MA) and migraine without aura (MO), consistently report a higher prevalence of migraine among depressed individuals compared to the general population (Breslau et al., 2000; Breslau et al., 2003; Merikangas et al., 1990; Merikangas et al., 1988). There is currently no verified explanation for this comorbidity, although it has been suggested that common biological pathways, such as the serotonergic and dopaminergic system may be involved (Breslau et al., 1991; Frediani & Villani, 2007). An important question that needs to be answered is whether depression is associated with a specific subtype or form of migraine. Several studies report that MA is more strongly correlated with depression than MO (Breslau et al., 2000; Merikangas et al., 1993; Mitsikostas & Thomas, 1999; Samaan et al., 2009). One interpretation of this finding is that migraine patients with comorbid depression suffer from a different type of migraine than 'pure' migraineurs, which causes them to experience more aura symptoms. Alternatively, however, this finding might indicate that individuals with more severe forms of migraine have a higher risk of developing depression. Given the symptomatic overlap between MO and MA, and the lack of evidence that these two disorders are etiologically distinct subtypes of migraine (Ligthart et al., 2006; Nyholt et al., 2004), this seems to be a plausible explanation.

To investigate whether depression is associated with a specific type of migraine, we reverse the question: are the migraines of depressed and non-depressed individuals similar in characteristics? If there are observable qualitative differences in the manifestation of migraine in depressed and non-depressed individuals, this may indicate there is a difference in the etiology of migraine in both groups. To address this issue, we compared migraine symptomatology in a large sample of MDD patients and a control sample, selected for low risk of depression. Using latent class analysis, individuals were empirically classified according to the pattern of headache symptoms they reported. Then the headache symptom profiles were compared between the MDD and the non-MDD group. Thus, qualitative differences in migraine symptomatology could be assessed while still allowing for anticipated differences in prevalence and severity.

METHODS

SAMPLE

The depressed sample in this study consisted of MDD cases diagnosed according to DSM-IV criteria (American Psychiatric Association, 2001) with the Composite International Diagnostic Interview (CIDI; Wittchen, 1994) The majority of MDD cases were originally recruited for the Netherlands Study of Depression and Anxiety (NESDA; Penninx et al., 2008). Of the 2981 NESDA participants, 2601 filled in a self-report questionnaire that provided information on migraine. Of these individuals, 1636 were diagnosed with lifetime MDD (1017 of whom had a diagnosis of MDD in the past year). All individuals with a lifetime diagnosis of MDD were included in this study. 756 were recruited through primary care, 561 through specialized mental health care and another 319 from the general population. Individuals who did not have a lifetime MDD diagnosis were not included. All NESDA participants underwent a 4-hour baseline assessment at one of seven clinic sites between September 2004 and February 2007. Part of this assessment were an interview on somatic health, functioning and health care use, and the administration of several written questionnaires (Licht et al., 2008), which included a section on migraine symptomatology (see below). A detailed description of sampling and ascertainment procedures for the NESDA study can be found elsewhere (Penninx et al., 2008).

The remainder of the study sample consisted of volunteer members of the Netherlands Twin Registry (NTR), based at the department of Biological Psychology at VU University in Amsterdam. In this group, the migraine data were collected as part of a longitudinal study on health, lifestyle and personality. The data used in the present study were collected in 2002 and 2004. Data collection procedures are described in detail elsewhere (Boomsma et al., 2006; Distel et al., 2007). These surveys included the same headache section that was included in the NESDA questionnaire. When a participant answered the headache section in both surveys, the most recent (2004) survey was used. Headache data were available for a total of 4047 families. In a subset of these families, one or more individuals had been diagnosed with MDD in an earlier study of anxious depression (Boomsma et al., 2000), based on a CIDI interview. In addition, an anxious depression factor score was constructed based on data from the 2002 survey, using several measures of anxiety, depression and neuroticism [see Boomsma et al. (2000) for details]. The 2004 survey included

the NEO Five Factor Inventory (Costa & McCrae, 1992), which has a neuroticism subscale.

NTR participants with a diagnosis of MDD based on the CIDI interview were included as additional MDD cases. In case of multiple individuals with MDD within a family, the individual with the highest anxious depression or neuroticism score was included. With this procedure an additional 180 MDD cases were selected, resulting in a total number of 1816 individuals with MDD.

The non-depressed control sample was also selected from the NTR, after excluding the families in which one or more individuals had been diagnosed as MDD cases. One person was selected from each family to maintain a selection of unrelated controls. Within each family, individuals were ranked based on their anxious depression score. This information was available for 3209 families. In families with no anxious depression scores available (N = 594), the neuroticism scale of the NEO-FFI was used. From each family, the individual with the lowest anxious depression or neuroticism score was selected. For a few families (N = 4) no information on anxious depression or neuroticism was available, in which case one individual was drawn at random. All individuals with an anxious depression or neuroticism score higher than one SD above the mean were excluded. This resulted in a low-risk control sample of 3428 individuals.

The control sample included 1379 male and 2049 female participants. The MDD sample included 553 males and 1263 females. The mean age was 42.6 (±12.4) in the MDD sample and 41.1 (±14.0) in the control sample.

Migraine measures

Migraine was assessed based on the International Classification of Headache Disorders (ICHD)-II criteria of the International Headache Society (IHS; Headache Classification Committee of the International Headache Society, 2004). Not only the endpoint diagnosis but especially its components (i.e. individual migraine symptoms/characteristics) were studied, to see whether 'symptom profiles' differed between the MDD group and the controls. The presence of these symptoms was assessed using questionnaire items which provided information on the IHS criteria for migraine. The headache section of the questionnaires was preceded by a screening question ("Do you ever experience headache attacks, for instance migraine?"). Individuals screening positive then answered the remaining questions. The questionnaire items are described in Table 6.1.

TABLE 6.1

Headache questions included in the surveys and correspondence to IHS diagnostic criteria for migraine		
Item in survey	**Code**	**Description**
Do you ever experience headache attacks, for instance migraine? (yes/no)	-	Screening question
How often do you have these headache attacks?*	A	>= 5 episodes
How long do these headache attacks usually last?	B	4-72 hours
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?†	D2	Photo and phonophobia
partial loss of vision, seeing flashes of light or (zigzag) patterns?	Aura	Visual aura
In the present study, individuals were considered positive for a full IHS migraine diagnosis if they fulfilled the following criteria: A; B; at least two of C2, C3 and C4; at least one of D1 and D2.		
* An attack frequency of 'several times a year' or more was assumed to be equivalent to '>= 5 episodes'.		
† 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.		

The information obtained from the questionnaire items was recoded as follows: 0 = screened negative, 1 = screened positive, but negative for symptom, 2 = screened positive, and positive for symptom. This was done for the variables *>= 5 episodes*, *4-72h* [duration], *pulsating*, *moderate/severe* [pain intensity], *aggravation* [by physical activity], *nausea/vomiting*, *photo-/phonophobia* and *(visual) aura*. These symptom variables were also used to establish a diagnosis according to the official IHS criteria (see Table 6.1).

Statistical analyses

Latent Class Analysis (LCA; see, e.g., Lazarsfeld & Henry, 1968; McCutcheon, 1987) is a statistical method that classifies individuals based on their pattern of responses or characteristics. A latent class model describes the relationship between a set of categorical observed variables (indicators) and an unobserved categorical variable. The categories of this underlying variable are referred to as latent classes, or clusters. Within each cluster, the observed variables are assumed to be independent. In other words, the relationship between the observed variables (in this case, migraine symptoms) is explained entirely by the latent variable (in this case, 'type of headache'). The parameters in an LCA model are the prevalence of each class, and the probability, given class membership, that an individual is positive for each symptom (the conditional probabilities). They are estimated with the expectation-maximization (EM) algorithm (Dempster et al., 1977). For each individual, the most likely class membership can then be calculated, based on the pattern of symptoms they report.

The aim of this study was to determine whether the same latent classes of headache sufferers could be identified in the MDD patients and the controls. We first estimated the number of latent classes present in the two samples. Then the symptom profiles of each group were compared by running a multiple-group LCA with the headache symptoms as the indicator variables. Differences in the symptom profiles were tested by equating the conditional probabilities for the classes across groups, and assessing the change in model fit by comparing log-likelihood values. Because migraine is known to be more prevalent in females than in males, it was first tested whether symptom profiles differed across sex. Next, profile differences between the MDD patients and the controls were assessed. Finally, classification results were compared between the two groups to test for differences in prevalence. All latent class analyses were performed in Mplus version 5, using the 'KNOWNCLASS' option to allow multi-group LCA. The number of random sets of starting values for the initial stage was set to 250

and in the final stage 50 maximum likelihood optimizations were specified. The number of classes was determined using the Bayes Information Criterion (BIC), with a lower BIC indicating a better fit to the data.

RESULTS

MDD patients showed a significantly higher prevalence of all migraine symptoms (Table 6.2). Generally, the symptom prevalence was two to three times higher in the MDD group than in the control group, confirming the strong association that exists between migraine and depression at the level of individual migraine symptoms. Table 6.2 also shows the number of individuals who would receive a full diagnosis of migraine (either MO or MA), according to IHS criteria. The number of full IHS migraine diagnoses is significantly higher in the MDD patients (22%) than in the controls (7%). The relationship between migraine and MDD was somewhat more pronounced for males than for females; generally, migraine symptoms had a 3-4 times higher prevalence in the depressed compared to the non-depressed males. In females the risk was about two times higher for the MDD group.

Initially, an exploratory latent class analysis was performed to determine the appropriate number of classes and to compare the symptom profiles in males and females. A two-group analysis was run with sex as the grouping ('KNOWNCLASS') variable, thus allowing for different symptom profiles in males and females. Sex was also modeled as a covariate on class membership, to allow for different migraine prevalences in males and females. This analysis was run first on cases only, and then on controls only. Based on the BIC values, a 3-class model had the best fit to the data in both the cases and the controls: in cases, the 3-class model produced a BIC of 13542, compared to a BIC of 13671 for a 2-class model and BIC of 13760 for a 4-class model; in controls, the 3-class model produced a BIC of 16112, compared to a BIC of 16209 for a 2-class and BIC of 16348 for a 4-class model.

Next, the conditional probabilities (i.e. the symptom profiles) were equated for males and females, assuming the 3-class model (Table 6.3). This did not result in a significant change in model fit in either cases or controls [$\chi^2(48) = 16.55$, $p = 1.000$ for cases, $\chi^2(48) = 38.29$, $p = .841$ for controls].

Since the symptom profiles did not differ between males and females we proceeded with a two-group model (with 3 classes) in which the conditional probabilities were equal for males and females but differed between the MDD and control group. Sex and case/control status were maintained in the model as covariates, because of the known differences in migraine prevalence across these groups. Figure 6.1 shows the symptom profiles for this model, with the symptoms on the x-axis, the conditional probabilities for each symptom on the y-axis and the error bars indicating 95% confidence intervals. Class 0 represents the group of individuals screening negative for headaches, who did not answer further questions. These individuals have conditional probabilities of 0 for all symptoms. Class 1 individuals have headaches with migrainous features, but most of these would not be diagnosed as migraine patients. The individuals in class 2 can be characterized as migrainous headache sufferers, with headaches that typically include the majority of migraine features. The most important difference between class 1 and 2 appears to be the overall severity of the headaches. Class 1 and 2 look similar, but all symptoms are more prevalent in class 2. The distinction between class 1 and 2 is most pronounced for the symptoms nausea/vomiting, photo-/phonophobia and aura. Of the individuals in class 1, 3% satisfied the IHS criteria for migraine (all MO). In class 2, 55% met these criteria (55% MO, and 40% MA and 5% unclassified due to missing aura data).

It can be seen that the profiles of MDD and non-MDD subjects are very similar, although some subtle differences are observed in the prevalence of aggravation, photo-/phonophobia and aura (only the estimates for aggravation and aura showed non-overlapping confidence intervals for the MDD and control groups). These symptoms had higher conditional probabilities in the MDD patients than in the controls in both class 1 (mild symptoms) and class 2 (severe symptoms). In class 1 the differences were more pronounced (with endorsement frequencies of 45% versus 64% for aggravation, and 15% versus 24% for aura, in non-MDD and MDD subjects, respectively) than in class 2 (83% versus 91% for aggravation and 42% versus 53% for aura). The overall significance of these profile differences was tested by equating the conditional probabilities for MDD patients and controls, which produced a significantly worse fit to the data ($\chi^2(48) = 145.67$, $p < .0001$).

TABLE 6.2

The prevalence of migraine symptoms and IHS migraine diagnosis in the depressed (MDD+) and non-depressed (MDD-) groups						
	MDD- (N = 1379)		MDD+ (N = 553)			
Males	**N**	**%**	**N**	**%**	**OR**	**95% CI**
SQ+	195	14%	241	44%	4.76	(3.80-5.97)
>= 5 episodes	159	12%	226	41%	5.30	(4.19-6.72)
4-72h	99	7%	109	20%	3.17	(2.37-4.25)
Pulsating	102	7%	116	21%	3.32	(2.49-4.43)
Moderate/ severe	158	11%	220	40%	5.11	(4.03-6.48)
Aggravation	100	7%	174	31%	5.87	(4.48-7.70)
Nausea/ vomiting	52	4%	67	12%	3.52	(2.41-5.13)
Photo-/ phonophobia	98	7%	146	26%	4.69	(3.55-6.20)
Aura	39	3%	92	17%	6.86	(4.65-10.12)
IHS migraine	42	3%	63	11%	4.09	(2.73-6.13)
	MDD- (N = 2049)		MDD+ (N = 1263)			
Females	**N**	**%**	**N**	**%**	**OR**	**95% CI**
SQ+	581	29%	735	60%	3.60	(3.10-4.18)
>= 5 episodes	536	26%	710	56%	3.62	(3.12-4.21)
4-72h	365	18%	447	35%	2.53	(2.15-2.97)
Pulsating	345	17%	379	30%	2.12	(1.79-2.50)
Moderate/ severe	556	27%	707	56%	3.41	(2.95-3.96)
Aggravation	404	20%	614	49%	3.85	(3.30-4.50)
Nausea/ vomiting	285	14%	341	27%	2.29	(1.92-2.73)
Photo-/ phonophobia	377	18%	550	44%	3.42	(2.92-4.01)
Aura	184	9%	306	24%	3.24	(2.66-3.95)
IHS migraine	199	10%	328	26%	3.26	(2.69-3.96)

Table 6.2 (continued)

	MDD- (N = 3428)		MDD+ (N = 1816)			
All	N	%	N	%	OR	95% CI
SQ+	776	23%	976	55%	4.06	(3.59-4.60)
>= 5 episodes	695	20%	936	52%	4.18	(3.69-4.74)
4-72h	464	14%	556	31%	2.82	(2.45-3.24)
Pulsating	447	13%	495	27%	2.50	(2.17-2.88)
Moderate/ severe	714	21%	927	51%	3.96	(3.50-4.49)
Aggravation	504	15%	788	43%	4.45	(3.90-5.08)
Nausea/ vomiting	337	10%	408	22%	2.66	(2.27-3.11)
Photo-/ phonophobia	475	14%	696	38%	3.86	(3.37-4.42)
Aura	223	7%	398	22%	4.03	(3.39-4.81)
IHS migraine	241	7%	391	22%	3.63	(3.05-4.31)

SQ+ = positive for screening question; OR = odds ratio indicating risk of each symptom/diagnosis, given depression status

TABLE 6.3

Model fit statistics and comparisons for the baseline and restricted 3-class LCA models									
	Model	N	npar	LL	Scaling correction factor	Compared to	X^2	df	p-value
1	male vs. female; different profiles, non-depressed cohort	3428	101	-7645.22	1.014				
2	male vs. female; equated profiles, non-depressed cohort	3428	53	-7664.55	1.018	1	38.29303	48	0.841
3	male vs. female; different profiles, depressed cohort	1816	101	-6392.11	1.014				
4	male vs. female; equated profiles, depressed cohort	1816	53	-6400.55	1.009	3	16.55287	48	1.000
5	depressed vs. non-depressed; different profiles	5244	103	-14024.1	1.013				
6	depressed vs. non-depressed; equated profiles	5244	55	-14097.8	1.013	6	145.6663	48	0.000

LCA = latent class analysis; npar = number of parameters; LL = Log-likelihood; df = degrees of freedom.

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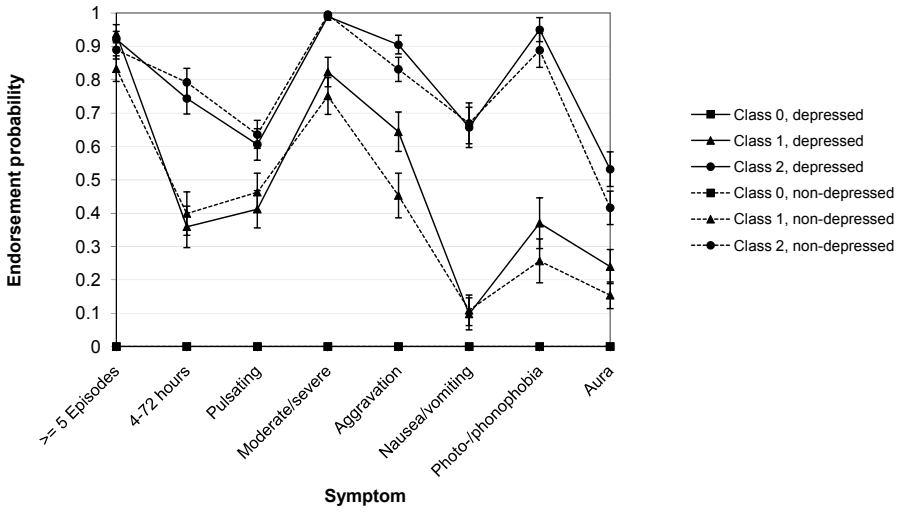

Figure 6.1

Symptom profiles based on the best-fitting 3-class LCA model. The error bars indicate 95% confidence intervals for the estimates of the endorsement probability. The profiles are plotted separately for the depressed and non-depressed groups.

Table 6.4 shows the classifications resulting from the best-fitting model, which assumes the same conditional probabilities for males and females but not for MDD and non-MDD individuals. In both sexes, class prevalence differed significantly across depression status ($\chi^2(2) = 202.707$, $p < .0001$ in males and $\chi^2(2) = 283.258$, $p < .0001$ in females). The prevalence of both class 1 and class 2 headaches was significantly higher in MDD patients than in controls.

Discussion

The aim of this study was to compare migraine symptom profiles in MDD patients and controls, empirically classified according to their pattern of headache symptoms. If similar headache classes and symptom profiles would arise empirically and independently in MDD patients and controls, this would be consistent with the hypothesis that we are observing the same disorder in the two groups. Substantial qualitative differences, however, would suggest a difference in etiology.

TABLE 6.4

Class prevalences in the four analysis groups [male/female, depressed/non-depressed], based on best-fitting model					
		MDD-		MDD+	
		N	class proportion	N	class proportion
Males	Class 0	1179	85.5%	306	55.3%
	Class 1	123	8.9%	161	29.1%
	Class 2	77	5.6%	86	15.6%
	Total	1379	100.0%	553	100.0%
Females	Class 0	1415	69.1%	497	39.4%
	Class 1	204	10.0%	262	20.7%
	Class 2	430	21.0%	504	39.9%
	Total	2049	100.0%	1263	100.0%
MDD+ = depressed, MDD- = non-depressed					

As expected, the prevalence of migraine was higher in MDD patients. Importantly, all migraine symptoms had an increased prevalence in the MDD group, and MDD patients were overrepresented in both the mild and severe migraine class. This is consistent with the literature on the comorbidity of migraine and MDD. Qualitatively, however, migraine was very similar in MDD patients and controls. Similar symptom profiles were observed in the two groups, although a few differences should be mentioned. The most pronounced difference between MDD and non-MDD subjects is in the higher prevalence of aggravation and visual aura among the depressed individuals. While it is possible that these reflect real qualitative differences, alternative explanations should be considered. Especially in the case of aggravation, it is plausible that MDD patients tend to experience their headaches as more aggravating than non-depressed subjects as a result of their mood disorder. The increased prevalence of visual aura seems less likely to be a side effect of altered mood. One possible explanation for the difference is that the questionnaire item that assessed aura, does not measure aura sufficiently well. It could be that some patients in fact report some phenomenon related to depression, rather than real aura

symptoms. An alternative explanation is that brain abnormalities associated with MDD might make an individual more susceptible to the phenomenon of cortical spreading depression, generally viewed as the mechanism underlying the migraine aura (Bigal et al., 2009; Cutrer & Huerter, 2007). This would not exclude the possibility that the migraine attack following the aura phase shows the same pattern of symptoms in MDD patients and controls.

Although the observed differences are small and subtle, they are significant (additional analyses in which the aggravation symptom was excluded from the model still produced significantly different profiles for the two groups). Therefore we cannot exclude the possibility that these are true qualitative differences between depressed and non-depressed subjects. Also, it should be noted that qualitative similarity of migraine in MDD patients and controls is consistent with, but does not prove, a shared etiology.

Strengths and limitations

To our knowledge, this is the first study in which migraine symptomatology in MDD patients and controls is compared while taking into account expected differences in prevalence and severity. Figure 6.1 shows that more severely affected patients have a higher probability of all symptoms, but in particular nausea/vomiting, photo-/phonophobia and aura. Therefore, if prevalence and severity are not accounted for, a higher prevalence of these symptoms in MDD patients could be mistaken for a qualitative difference, whereas in reality it reflects a difference in the prevalence of severe migraine.

Another major strength of this study is the sample size, which is quite large compared to other studies of the comorbidity of migraine and depression. A total of 1816 clinically diagnosed MDD patients and 3428 controls selected for low risk of MDD participated, all of whom provided detailed information on migraine symptomatology.

At the same time, however, one potential limitation of this study is related to the sample size. Although the results of the latent class analyses in this study show considerable similarity to those we reported in previous studies (Ligthart et al., 2006; Nyholt et al., 2004), in previous studies the best fitting model was a 4-class rather than a 3-class model. This is almost certainly a consequence of the larger sample sizes in these studies, which allowed the distinction of a fourth class. However, the additional class estimated in the previous studies reflected a less severe, non-migrainous form of headache on the same continuum of liability, and as noted in our previous twin studies, a 3-class model captures most of the variance in migraine status that is captured by a 4-class model. In

addition, given that the current sample size (1816 cases and 3428 controls) is still quite large, any qualitative differences that can only be detected in larger samples would most likely be of little practical importance in distinguishing between 'pure migraine' and MDD-related migraine.

A second limitation concerns the questionnaire. Since no information was available on unilaterality of the headache, we cannot exclude the possibility that the frequency of unilateral headache may be different in MDD patients and controls. However, because patients were required to have at least 2 out of the 3 measured C criteria (see Table 6.1) to receive a migraine diagnosis, it is unlikely that the lack of information on unilateral headache has caused false positive endpoint diagnoses. Indeed, the observed prevalence of migraine according to ICHD-II criteria was 3% (males) and 10% (females) in the low-risk control sample, and 4% (males) and 13% (females) in the total, unselected NTR sample (N = 12,303), which is slightly lower than in other studies (Stewart et al., 1992), possibly due to our somewhat conservative definition of migraine. Also, in the total, unselected NTR-sample, 71% screened negative, which is relatively high compared to other studies (Nyholt et al., 2004). This suggests the screening procedure was somewhat strict. The screening question ("Do you ever experience headache attacks, for instance migraine?") did not specify a time frame; thus, attacks or symptoms that occur with a low frequency might not be reported. In addition, the phrasing might cause individuals who do not think their headache qualifies as migraine to respond negatively, even if they have some symptoms of migrainous headache. This would most likely result in an underestimate of the number of class 1 individuals. However, this issue is expected to affect prevalence estimates rather than estimates of qualitative migraine features.

Generalizability

The NTR is a population-based registry of unselected twin families. A non-response study found no evidence that participants' willingness to participate was related to migraine status (Distel et al., 2007). Whether findings in twins can be generalized to the singleton population can be tested by including data from the twins' siblings. In this study, twins had the same prevalence of each class of migrainous headache as their singleton siblings ($\chi^2(2) = 1.617$, $p = .446$). The MDD cases in the NESDA study were selected from three different settings (community, primary care, and specialized mental health care), to ensure that the resulting sample was representative of MDD in a wide range of settings, and included both milder and severe cases (Penninx et al., 2008). To

test whether the selection from different settings might have influenced the results, symptom profiles were estimated separately for MDD patients from each setting. No qualitative differences in the symptom profiles were found between settings. Finally, to test whether treatment of MDD might cause any qualitative changes in migraine symptomatology, profiles were estimated separately for the MDD patients who received psychotherapy or treatment with antidepressants (N = 871) and those who did not (N = 765). The symptom profiles showed no significant qualitative differences related to treatment.

Conclusions and implications

Two important observations were made in this study. Firstly, the prevalence of *all* migraine symptoms is dramatically increased in MDD patients compared to non-depressed controls. This is also reflected in the fact that comparatively more MDD patients are classified as class 1 and 2 migrainous headache sufferers. Interestingly, the relationship between migraine and MDD appears to be stronger in males than in females.

A second important observation is that the migraine symptom profiles of MDD patients and non-depressed controls are very similar, suggesting a similar disease process underlies migraine in both groups. We observed a slightly increased prevalence of aggravation and aura symptoms in the MDD group. However, the small size of the differences, combined with the large variability in symptoms among individual migraineurs indicate that looking at migraine symptoms alone does not support a distinction between 'pure' migraine and migraine associated with depression.

This highlights the importance of collecting additional information besides those that make up the official diagnostic criteria for a given disorder. Information on the presence of comorbid MDD (symptoms) may be vital for any study investigating the etiology of migraine. This may also extend to other traits. Many disorders show comorbidity with migraine, in particular psychiatric disorders (depression, anxiety, bipolar disorder, phobias and panic disorder; Breslau & Davis, 1992; Breslau et al., 1991; Merikangas et al., 1990) but also non-psychiatric disorders such as stroke, asthma, epilepsy, endometriosis and other chronic pain conditions (Anttila et al., 2001; Hagen et al., 2002; Merikangas & Stevens, 1997; Nyholt et al., 2009; Ottman & Lipton, 1994; Terwindt et al., 2000; Von Korff et al., 2005). Similarly, depressed individuals show an increased prevalence of a variety of somatic symptoms, compared to non-depressed subjects (Katona et al., 2005), and a recent study demonstrated that migraine was an important predictor of other somatic symptoms in depressed subjects

(Hung et al., 2009). In this context, it is interesting to mention the reported comorbidity between MDD and general chronic pain (Bair et al., 2003). Indeed, the MDD patients from the NESDA study reported a remarkably high frequency of pain symptoms, often at multiple sites (in the NTR these data were not available). While this might reflect a general tendency of depressed patients to more easily endorse questions regarding somatic complaints, it has been suggested that chronic pain might in fact be a symptom of depression (Lépine & Briley, 2004). Although beyond the scope of the present study, this is a fundamental issue with important implications for research on migraine comorbidity. In conclusion, the collection of extensive and detailed information on comorbid disorders in studies of migraine could potentially improve our understanding of the etiology of these disorders and may contribute towards a more effective study of their underlying causes.

References

American Psychiatric Association (2001). Diagnostic and Statistical Manual of Mental Disorders (4th ed.). Washington, DC: American Psychiatric Publishing.

Anttila, P., Metsahonkala, L., Mikkelsson, M., Helenius, H., & Sillanpaa, M. (2001). Comorbidity of other pains in schoolchildren with migraine or nonmigrainous headache. J Pediatr, 138(2), 176-180.

Bair, M. J., Robinson, R. L., Katon, W., & Kroenke, K. (2003). Depression and pain comorbidity: a literature review. Arch Intern Med, 163(20), 2433-2445.

Bigal, M. E., Ferrari, M., Silberstein, S. D., Lipton, R. B., & Goadsby, P. J. (2009). Migraine in the triptan era: lessons from epidemiology, pathophysiology, and clinical science. Headache, 49 Suppl 1, S21-33.

Boomsma, D. I., Beem, A. L., van den Berg, M., Dolan, C. V., Koopmans, J. R., Vink, J. M., et al. (2000). Netherlands twin family study of anxious depression (NETSAD). Twin Res, 3(4), 323-334.

Boomsma, D. I., de Geus, E. J., Vink, J. M., Stubbe, J. H., Distel, M. A., Hottenga, J. J., et al. (2006). Netherlands Twin Register: from twins to twin families. Twin Res Hum Genet, 9(6), 849-857.

Breslau, N., & Davis, G. C. (1992). Migraine, major depression and panic disorder: a prospective epidemiologic study of young adults. Cephalalgia, 12(2), 85-90.

Breslau, N., Davis, G. C., & Andreski, P. (1991). Migraine, psychiatric disorders, and suicide attempts: an epidemiologic study of young adults. Psychiatry Res, 37(1), 11-23.

Breslau, N., Schultz, L. R., Stewart, W. F., Lipton, R. B., Lucia, V. C., & Welch, K. M. (2000). Headache and major depression: is the association specific to migraine? Neurology, 54(2), 308-313.

Breslau, N., Schultz, L. R., Welch, K. M. A., Lipton, R. B., & Stewart, W. F. (2003). Comorbidity of migraine and depression: investigating potential etiology and prognosis. Neurology, 60(8), 1308-1312.

Costa, P. T., & McCrae, R. R. (1992). Revised NEO Personality Inventory (NEO-PI-R) and NEO Five-Factor Inventory (NEO-FFI); Professional Manual. Odessa, FL: Psychological Assessment Resources.

Cutrer, F. M., & Huerter, K. (2007). Migraine aura. Neurologist, 13(3), 118-125.

Dempster, A. P., Laird, N. M., & Rubin, D. B. (1977). Maximum Likelihood from Incomplete Data Via Em Algorithm. Journal of the Royal Statistical Society Series B-Methodological, 39(1), 1-38.

Distel, M. A., Ligthart, L., Willemsen, G., Nyholt, D. R., Trull, T. J., & Boomsma, D. I. (2007). Personality, Health and Lifestyle in a Questionnaire Family Study: A Comparison

Between Highly Cooperative and Less Cooperative Families. Twin Res Hum Genet, 10(2), 348-353.

Frediani, F., & Villani, V. (2007). Migraine and depression. Neurol Sci, 28 (Suppl 2), S161-165.

Hagen, K., Einarsen, C., Zwart, J. A., Svebak, S., & Bovim, G. (2002). The co-occurrence of headache and musculoskeletal symptoms amongst 51 050 adults in Norway. Eur J Neurol, 9(5), 527-533.

Headache Classification Committee of the International Headache Society (2004). The International Classification of Headache Disorders: 2nd edition, Cephalalgia (Vol. 24 [Suppl 1], pp. 9-160).

Hung, C. I., Liu, C. Y., Cheng, Y. T., & Wang, S. J. (2009). Migraine: A missing link between somatic symptoms and major depressive disorder. J Affect Disord.

Katona, C., Peveler, R., Dowrick, C., Wessely, S., Feinmann, C., Gask, L., et al. (2005). Pain symptoms in depression: definition and clinical significance. Clin Med, 5(4), 390-395.

Lazarsfeld, P. F., & Henry, N. W. (1968). Latent structure analysis. New York: Houghton, Mifflin.

Lépine, J. P., & Briley, M. (2004). The epidemiology of pain in depression. Hum Psychopharmacol, 19 Suppl 1, S3-7.

Licht, C. M., de Geus, E. J., Zitman, F. G., Hoogendijk, W. J., van Dyck, R., & Penninx, B. W. (2008). Association between major depressive disorder and heart rate variability in the Netherlands Study of Depression and Anxiety (NESDA). Arch Gen Psychiatry, 65(12), 1358-1367.

Ligthart, L., Boomsma, D. I., Martin, N. G., Stubbe, J. H., & Nyholt, D. R. (2006). Migraine with aura and migraine without aura are not distinct entities: further evidence from a large Dutch population study. Twin Res Hum Genet, 9(1), 54-63.

McCutcheon, A. L. (1987). Latent class analysis. Beverly Hills: Sage Publications.

Merikangas, K. R., Angst, J., & Isler, H. (1990). Migraine and psychopathology. Results of the Zurich cohort study of young adults. Arch Gen Psychiatry, 47(9), 849-853.

Merikangas, K. R., Merikangas, J. R., & Angst, J. (1993). Headache syndromes and psychiatric disorders: association and familial transmission. J Psychiatr Res, 27(2), 197-210.

Merikangas, K. R., Risch, N. J., Merikangas, J. R., Weissman, M. M., & Kidd, K. K. (1988). Migraine and depression: association and familial transmission. J Psychiatr Res, 22(2), 119-129.

Merikangas, K. R., & Stevens, D. E. (1997). Comorbidity of migraine and psychiatric disorders. Neurol Clin., 15(1), 115-123.

Mitsikostas, D. D., & Thomas, A. M. (1999). Comorbidity of headache and depressive disorders. Cephalalgia, 19, 211-217.

Nyholt, D. R., Gillespie, N. G., Heath, A. C., Merikangas, K. R., Duffy, D. L., & Martin, N. G. (2004). Latent class and genetic analysis does not support migraine with aura and migraine without aura as separate entities. Genet Epidemiol, 26(3), 231-244.

Nyholt, D. R., Gillespie, N. G., Merikangas, K. R., Treloar, S. A., Martin, N. G., & Montgomery, G. W. (2009). Common genetic influences underlie comorbidity of migraine and endometriosis. Genet Epidemiol, 33(2), 105-113.

Ottman, R., & Lipton, R. B. (1994). Comorbidity of migraine and epilepsy. Neurology, 44(11), 2105-2110.

Penninx, B. W., Beekman, A. T., Smit, J. H., Zitman, F. G., Nolen, W. A., Spinhoven, P., et al. (2008). The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res, 17(3), 121-140.

Samaan, Z., Farmer, A., Craddock, N., Jones, L., Korszun, A., Owen, M., et al. (2009). Migraine in recurrent depression: case-control study. Br J Psychiatry, 194(4), 350-354.

Stewart, W. F., Lipton, R. B., Celentano, D. D., & Reed, M. L. (1992). Prevalence of migraine headache in the United States. Relation to age, income, race, and other sociodemographic factors. Jama, 267(1), 64-69.

Terwindt, G. M., Ferrari, M. D., Tijhuis, M., Groenen, S. M., Picavet, H. S., & Launer, L. J. (2000). The impact of migraine on quality of life in the general population: the GEM study. Neurology, 55(5), 624-629.

Von Korff, M., Crane, P., Lane, M., Miglioretti, D. L., Simon, G., Saunders, K., et al. (2005). Chronic spinal pain and physical-mental comorbidity in the United States: results from the national comorbidity survey replication. Pain, 113(3), 331-339.

Wittchen, H. U. (1994). Reliability and validity studies of the WHO--Composite International Diagnostic Interview (CIDI): a critical review. J Psychiatr Res, 28(1), 57-84.

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The shared genetics of migraine and anxious depression

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ABSTRACT

Objectives: To investigate 1) whether shared genetic factors influence migraine and anxious depression, 2) whether the genetic architecture of migraine depends on anxious depression, and 3) whether the association between the traits is causal. Background: Migraine and anxious depression frequently co-occur, but little is known about the mechanisms causing this association. ***Methods:*** A twin study was conducted to model the genetic architecture of migraine and anxious depression and the covariance between them. Anxious depression was also added to the model as a moderator variable to examine whether anxious depression affects the genetic architecture of migraine. Causal models were explored with the co-twin control method. ***Results:*** Modest but significant phenotypic (rP = .28), genetic (rG = .30) and non-shared environmental (rE = .26) correlations were found between the two traits. Interestingly, the heritability of migraine depended on the level of anxious depression: migraine was less heritable in subjects with high anxious depression scores. The observed risk patterns in discordant twins are most consistent with a bidirectional causal relationship. ***Conclusions:*** These findings confirm the genetic association between migraine and anxious depression and are consistent with a syndromic association between the two traits. This highlights the importance of taking comorbidity into account in genetic studies of migraine, especially in the context of selection for large-scale genotyping efforts. Genetic studies may be most effective when migraine with and without comorbid anxious depression are treated as separate phenotypes.

Introduction

Migraine and depression consistently show an association, which may be explained by a shared etiology, for instance, genetic risk factors. Several authors have suggested that disturbances in the serotonergic and dopaminergic systems, involved in both migraine and depression, might explain the association between the two traits (Breslau et al., 1991; Frediani & Villani, 2007).

Two recent studies investigated the association between migraine and depression and found that the two traits were genetically correlated (Schur et al., 2009; Stam et al., 2010). This may reflect the existence of genetic risk factors that can cause migraine as well as depression (pleiotropy). Alternatively, if there is a causal relationship between two traits, genetic factors contributing to the first trait will also explain variance in the second trait. Thus, a causal relationship is also consistent with a genetic correlation. Whether traits are related causally or through an underlying shared etiology, can be examined using family data (Kendler et al., 1993; Merikangas & Stevens, 1997).

In the present study, we investigated the shared genetics of migraine and anxious depression in three different ways. A twin design was used to (1) test whether the previously reported genetic correlation between migraine and depression could be replicated in migraine and anxious depression data from a large number of Dutch twins; (2) investigate whether the genetic architecture of migraine was the same in individuals with high and low anxious depression scores. Finally, to address the question of causality, the co-twin control method (Kendler et al., 1993) was applied to investigate whether the association between migraine and anxious depression is more likely explained by a causal model or a shared underlying etiology.

Methods

Subjects

The participants in this study were volunteer members of the Netherlands Twin Registry (NTR), based at the department of Biological Psychology of the VU University in Amsterdam. NTR participants receive mailed questionnaires every two to three years, in the context of an ongoing study of health, lifestyle and personality. The migraine and anxious depression data used in this study were collected in the 2002 and 2004 surveys. When a participant answered the headache section in both surveys, the most recent (2004) survey was used. Data

collection procedures are described in detail elsewhere (Boomsma et al., 2006; Distel et al., 2007). The study was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Center, Amsterdam. All subjects provided written informed consent.

The analysis performed to assign affection status for migraine to each individual was based on the largest possible sample with migraine data available, including twins, parents, singleton siblings and spouses [N = 14,904, including 12,303 participants from the NTR and 2,601 from NESDA (Penninx et al., 2008)]. Further analyses were based on the data of twins only (N = 5,535; 2,072 complete pairs and 1,391 individuals from incomplete pairs). Migraine data were available for all 5,535 individuals; 4,320 twins also provided data on anxious depression, resulting in a total of 1,491 complete twin pairs with information on both migraine and anxious depression (223 monozygotic (MZ) male, 100 dizygotic (DZ) male, 602 MZ female, 286 DZ female, and 280 DZ opposite sex pairs). In total, the sample consisted of 1,774 (32%) male and 3,761 (68%) female participants and the mean age was 34.33 years (SD = 11.35, range 14-86 years).

MEASURES

The subjects completed a questionnaire that included items relating to the diagnostic criteria for migraine of the International Headache Society (Headache Classification Committee of the International Headache Society, 2004; see Table 7.1). Migraine status was assigned to each subject based on a latent class analysis (LCA; Lazarsfeld & Henry, 1968; McCutcheon, 1987), which empirically classifies individuals according to their pattern of reported migraine symptoms. For simplicity, LCA-derived migrainous headache will be referred to as 'migraine' throughout the remainder of the paper. The application of LCA in migraine studies has been described in more detail elsewhere (Ligthart et al., 2006; Ligthart et al., 2008; Nyholt et al., 2004; Nyholt et al., 2005). LCA was performed in Latent Gold 4.0 (Statistical Innovations Inc., Belmont, MA). The correct number of classes was determined based on the Bayes Information Criterion (BIC; Schwarz, 1978) with a lower BIC indicating a better fit to the data.

Table 7.1

Headache questions included in the surveys and correspondence to IHS diagnostic criteria for migraine		
Item in survey	**Code**	**Description**
Do you ever experience headache attacks, for instance migraine? (yes/no)	-	Screening question
How often do you have these headache attacks?*	A	>= 5 episodes
How long do these headache attacks usually last?	B	4-72 hours
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?†	D2	Photo- and phonophobia
partial loss of vision, seeing flashes of light or (zigzag) patterns?	Aura	Visual aura

* An attack frequency of 'several times a year' or more was assumed to be equivalent to '>= 5 episodes'.

† 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.

The anxious depression measure consisted of a factor score based on several measures of anxiety, depression and neuroticism that was calculated using an algorithm developed in previous research on anxious depression (Boomsma et al., 2000). This factor score was recoded into quartiles, with quartile 1 indicating a low anxious depression score and quartile 4 indicating a high score.

Genetic modeling

In the classical twin study, the resemblance between twins is used to estimate to what extent a trait is influenced by additive genetic factors (A), shared (or common) environment (C) and non-shared environment (E). MZ twins share 100% of their segregating genes, whereas DZ twins share on average 50%. Differences between MZ twins reflect E. Greater resemblance in MZ compared to DZ twins reflects genetic influences, with an MZ correlation (rMZ) equal to twice the DZ correlation (rDZ) indicating A, and an rMZ which is less than twice the rDZ indicating A and C. Based on these principles, the total variance in a trait can be decomposed into variance due to A, C and E. Estimation of the relative contributions of A, C and E can be accomplished with structural equation modeling (SEM). Figure 7.1 shows a path diagram of the model tested here. Since there was no evidence for shared environmental effects based on the observed twin correlations or the literature (Mulder et al., 2003; Sullivan et al., 2000), an AE model was tested for both traits.

To investigate whether the genetic and environmental factors influencing migraine and anxious depression were correlated, a bivariate genetic model was tested (Figure 7.1). This model included genetic and environmental factors for both traits, partly unique to each trait (the a_{11}, a_{22} e_{11} and e_{22} paths), and partly shared (a_{21} and e_{21}). The shared part represents the covariance between the two traits, which can be decomposed into covariance explained by genetic and environmental factors. This is done based on the cross-trait cross-twin correlations (i.e. the correlation between one trait in the first twin and the other trait in the second twin). The cross-twin cross-trait correlations are interpreted in the same way as the within-trait twin correlations, with correlations higher in MZ than DZ twins indicating genetic factors influencing both traits. By standardizing the parts of the covariance due to A and E, genetic and environmental correlations can be calculated. The significance of these correlations was tested by dropping the a_{21} and e_{21} paths from the model and comparing the fit of the restricted and full models.

A liability threshold model was tested for both migraine and anxious depression. A threshold model assumes that the observed categorical data (e.g.,

a variable with values 1-4 indicating severity of migraine) are an imperfect measurement of an underlying normal distribution of liability with a mean of zero and a variance of one. This distribution is divided into discrete categories by one or more threshold values, expressed as *Z*-scores. The area under the curve between two thresholds represents the prevalence of each category. The categorized anxious depression variable was already adjusted for sex; therefore the thresholds for both sexes were equated in the model. Migraine, as expected, had a higher prevalence in females. Thus, the thresholds for migraine were estimated separately for males and females.

To test whether the heritability of migraine depends on anxious depression, anxious depression was specified as a moderator of the path coefficients a_{21} and e_{21} (which represent the variance shared by migraine and depression) and a_{22}, and e_{22} (which represent the variance unique to migraine). In other words, the effects of the genetic and environmental factors affecting migraine were allowed to vary depending on depression status. The significance of the moderation effect was evaluated by dropping the beta parameters β_{AC}, β_{AU}, β_{EU} and β_{EC} from the model and assessing the difference in model fit.

To ensure identification of the model, the total variance in a threshold model has to be constrained to one. However, in the model used here the variance of migraine depends on the value of the moderator (anxious depression). Therefore, the moderator variable was converted to a *Z*-score; the variance was constrained to be one at the mean value of the moderator, as proposed by Medland and colleagues (Medland et al., 2009). All genetic modeling was performed in Mx (Neale et al., 2003).

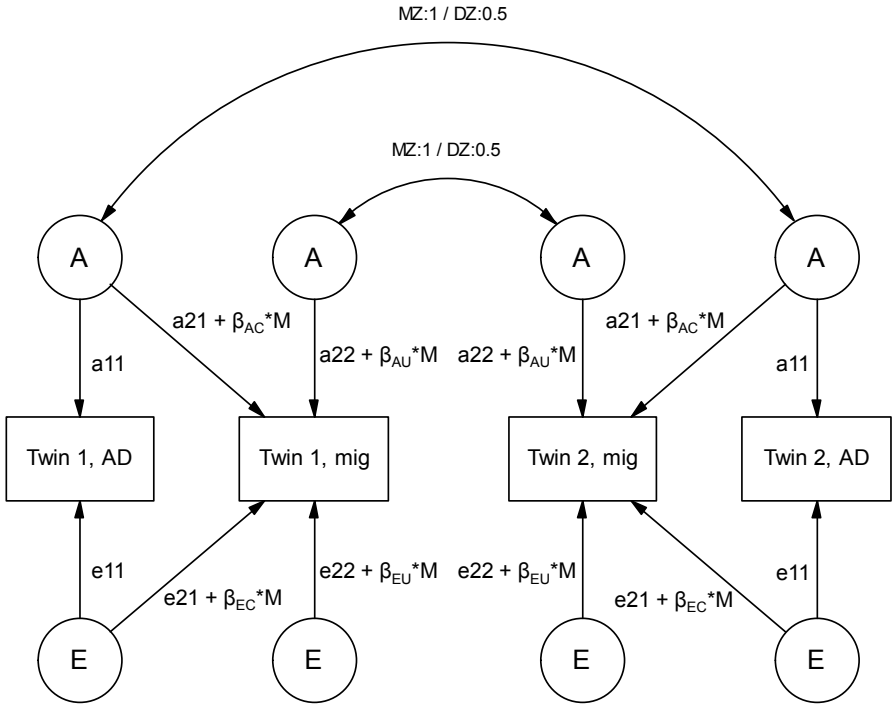

Figure 7.1

The bivariate moderator model. AD = anxious depression, mig = migraine. The A and E factors influencing migraine are moderated by anxious depression (M). Regression betas are estimated for the genetic factors unique to migraine (β_{AU}) and common to migraine and anxious depression (β_{AC}), and the same for the non-shared environmental factors (β_{EU} and β_{EC}). The moderator variable (anxious depression) affects both the variance unique to migraine (path coefficients a_{22} and e_{22}) and the variance shared with anxious depression (path coefficients a_{21} and e_{21}).

Co-twin control method

The co-twin control method (Kendler et al., 1993) was applied to test the hypothesis that 1) migraine causes anxious depression, and 2) anxious depression causes migraine. In this design, an odds ratio (OR) is calculated for trait A, given the presence or absence of trait B. This is done in three groups of individuals: MZ and DZ twin pairs discordant for trait B, and a case-control population sample. Under a *causal* model, all three groups are expected to show a similarly increased prevalence of A, given the presence of B, i.e., all three groups will have an OR > 1. Under a *non-causal* model, where shared underlying genetic factors explain the association, the OR in MZ twins is expected to equal one, because MZ twins are exposed to the same genetic risk factors, and should therefore have the same risk of trait A regardless of the presence of trait B. DZ twins will show an intermediate pattern (Figure 7.2).

For this analysis, anxious depression was dichotomized; individuals in the highest scoring quartile were treated as cases, the lowest three quartiles were treated as controls. A 'general population' sample was obtained by randomly selecting one individual from each family in the NTR sample (total N = 12,303), excluding the discordant twins. The sample included 358 MZ and 418 DZ pairs discordant for anxious depression, and 454 MZ and 510 DZ pairs discordant for migraine. The general population sample consisted of 2,838 unrelated individuals. ORs were calculated in SPSS 17.

Results

Four classes of individuals were identified, based on the patterns of reported migraine symptoms. The 4-class LCA model provided a better fit to the data (BIC = 60139.87) than a 3 or a 5-class model (with a BIC of 60185.03 and 60233.40, respectively). Figure 7.3 shows the pattern of symptoms in each class. The two most severe classes were treated as affected for migrainous headache, the remaining individuals were treated as unaffected. In the twin sample used in all subsequent analyses, 14% of the male and 35% of the female participants were classified as affected, which is comparable to the combined prevalence of migraine and probable migraine, according to IHS criteria (Merikangas et al., 1990).

A clear comorbidity of migraine and depression was observed, with a migraine prevalence of 20% in the lowest anxious depression quartile and 43% in the highest scoring quartile. The phenotypic correlation between migraine and anxious depression was estimated at .28 (95% CI = .20 - .36).

Table 7.2 shows an overview of the correlations across twins and traits. The twin correlations for both migraine and anxious depression were clearly higher in MZ than DZ twins, reflecting genetic influences on both traits. Genetic modeling results indicated that the variance in migraine could be explained by a combination of genetic (45%) and non-shared environmental factors (55%). For anxious depression, genetic factors explained 55% and non-shared environment explained 45% of the variance.

TABLE 7.2

Correlation matrices for MZ and DZ twins							
MZ	**AD twin 1**	**95% CI**	**mig twin 1**	**95% CI**	**AD twin 2**	**95% CI**	**mig twin 2**
AD twin 1	1.00						
mig twin 1	0.28	(.20-.36)	1.00				
AD twin 2	0.55	(.49-.60)	0.15	(.08-.22)	1.00		
mig twin 2	0.15	(.08-.22)	0.45	(.35-.55)	0.28	(.20-.36)	1.00
DZ	**AD twin 1**	**95% CI**	**mig twin 1**	**95% CI**	**AD twin 2**	**95% CI**	**mig twin 2**
AD twin 1	1.00						
mig twin 1	0.28	(.20-.36)	1.00				
AD twin 2	0.27	(.25-.30)	0.08	(.04-.11)	1.00		
mig twin 2	0.08	(.04-.11)	0.23	(.18-.27)	0.28	(.20-.36)	1.00

MZ = monozygotic, DZ = dizygotic, AD = anxious depression, mig = migraine

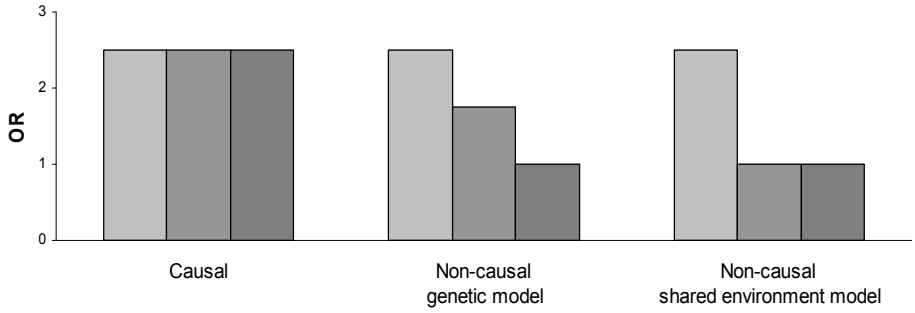

Figure 7.2

Expected patterns of odds ratios (OR) for general population and discordant DZ and MZ twins under the assumptions of causality and non-causality. Under the causal hypothesis, trait A and B are associated in all three groups. Under the non-causal hypothesis, where genetic factors explain the association, discordant MZ twins have an OR of 1, because they are genetically identical and are thus exposed to the same genetic risk factors. The DZ twins, who share on average 50% of their segregating genes, show an intermediate pattern. Finally, if the association is non-causal but explained by shared environment, all discordant twins are expected to have an OR of one. However, in this case, this is unlikely because there is no evidence that shared environment affects migraine or depression.

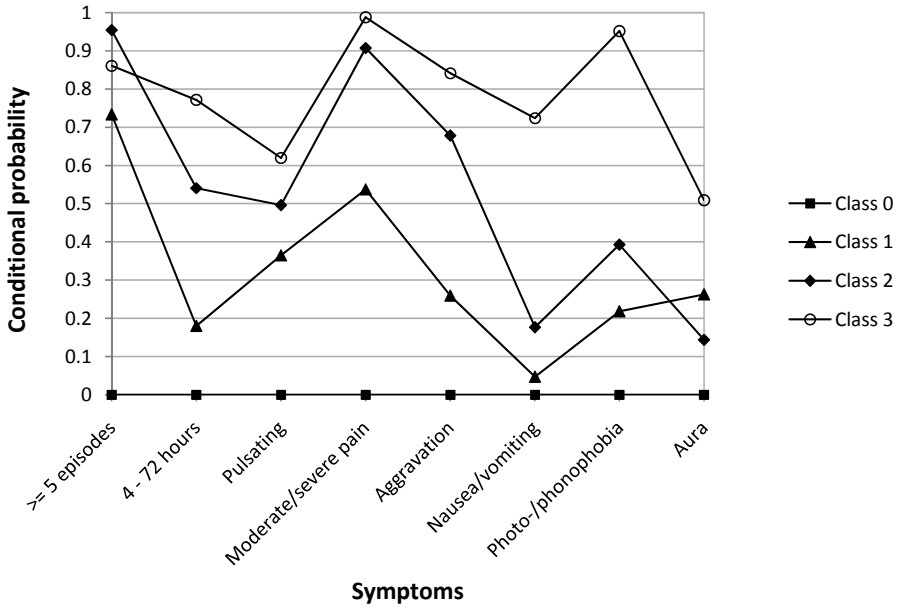

FIGURE 7.3

Profile plot for the best fitting latent class model, showing the symptom prevalence in each of the empirically estimated classes. The migraine symptoms are on the x-axis, the y-axis shows the probability that a symptom is present given class membership.

The cross-twin cross-trait correlations were also higher in MZ than DZ twins, suggesting the correlation between migraine and anxious depression is at least partly explained by genetic influences. Most of the covariance between the two traits was indeed explained by shared genetic factors (54%), while non-shared environment was responsible for the remaining covariance (46%). The genetic correlation (rG) between anxious depression and migraine was estimated at .30 (95% CI = .18 - .43) while the non-shared environmental correlation (rE) was .26 (95% CI = .15 - .37). Both correlations were significant: dropping a_{21} and e_{21} from the model both resulted in a significant deterioration in model fit ($\Delta\chi^2(1) = 17.834$, $p < .001$ for a_{21}, $\Delta\chi^2(1) = 15.535$, $p < .001$ for e_{21}).

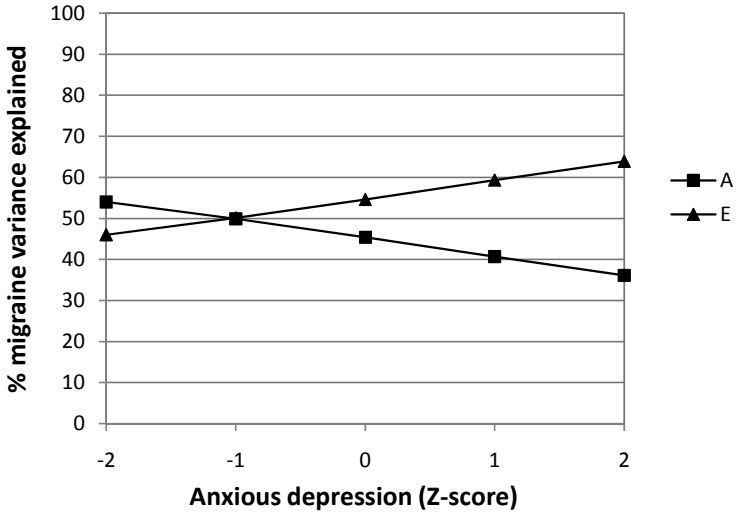

Figure 7.4

The heritability of migraine at different values of anxious depression. The proportion of variance in migraine explained by additive genetic factors (A) and non-shared environmental factors (E) across a range of depression scores, based on the estimates obtained from the moderator model. The higher the depression score, the lower the relative contribution of genetic factors to the individual differences in migraine susceptibility. Low = anxious depression score 2 SD below the mean, high = anxious depression score 2 SD above the mean.

The next step was to test the significance of the moderation effect of anxious depression on the heritability of migraine, by dropping the moderator betas from the model and assessing the resulting deterioration in model fit. The power to test the significance of these parameters individually was low (as reflected by confidence intervals that included zero; Table 7.3). However, dropping all four β parameters from the model at once resulted in a significant deterioration of the model fit [$\Delta\chi^2$ (4) = 12.478, p = .014], indicating that, overall, the moderator variable is of importance in explaining the observed data. Figure 7.4 shows the effect of anxious depression on the genetic and environmental factors influencing migraine. The heritability of migraine was lower at a higher level of anxious depression. In other words, migraine is most heritable in the absence of anxious depression.

TABLE 7.3

Point estimates for the parameters that constitute the variance in migraine		
parameter	point estimate	95% CI
a_{21}	0.21	(0.11 - 0.30)
a_{22}	0.64	(0.56 - 0.71)
e_{21}	0.19	(0.10 - 0.29)
e_{22}	0.71	(0.64 - 0.78)
β_{EC}	0.07	(-0.03 - 0.16)
β_{EU}	0.00	(-0.13 - 0.12)
β_{AC}	0.04	(-0.05 - 0.13)
β_{AU}	-0.07	(-0.19 - 0.06)

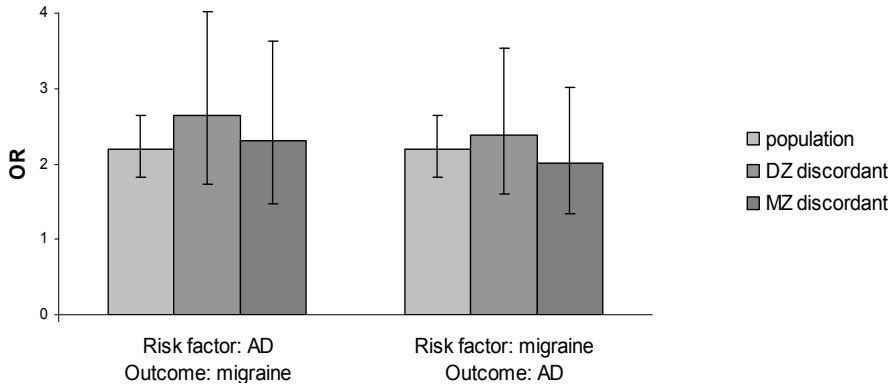

FIGURE 7.5

Observed pattern of odds ratios (OR) in general population and discordant DZ and MZ twin pairs, for both possible directions of causality between migraine and anxious depression (AD). The error bars represent the 95% confidence intervals around the ORs. In both situations, all ORs are significantly larger than 1, and have roughly the same size for each group. This is most consistent with the causal hypothesis and excludes an entirely non-causal hypothesis, because in that case the OR for MZ twins would not be significantly larger than one (see Figure 7.2).

Finally, Figure 7.5 shows the results of the co-twin control analysis. The OR is roughly the same for MZ, DZ and general population, under both hypotheses (migraine causes anxious depression and anxious depression causes migraine). The 95% confidence intervals indicate that both in MZ and DZ discordant twin pairs the ORs were significantly larger than 1. These results are most consistent with a bidirectional causal relationship between migraine and anxious depression.

Discussion

The results of this study are interesting in several aspects. First, they confirm the presence of a genetic correlation between migraine and anxious depression. This is consistent with the findings of two other recent studies on this topic (Schur et al., 2009; Stam et al., 2010).

A second important outcome of this study is that migraine was more heritable when not accompanied by comorbid depression. A possible explanation for this finding would be that some neurological disturbance in the brain, associated with depression, also makes patients more vulnerable to migraine. Thus, depressed individuals without a severe genetic predisposition to migraine might still develop migraine attacks regularly. Clearly, this theory is speculative and needs further investigation; interestingly, however, various studies have shown that depressed patients report several different types of pain (headache, low back pain, abdominal pain, etc.) more frequently than non-depressed individuals, suggesting that depression increases an individual's vulnerability to pain conditions (Bair et al., 2003). It has been argued that pain should in fact be considered a symptom of depression (Lépine & Briley, 2004). It is unclear whether there is a specific association of depression with migraine (beyond the general increase in pain symptoms associated with depression), because to date, studies of migraine and depression have not accounted for the phenomenon of comorbid pain in depressed individuals.

A third important finding is that migraine and depression are most likely causally related in two directions. In MZ twin pairs discordant for anxious depression, the non-depressed twin did not have an increased risk of migraine, and in MZ twin pairs discordant for migraine, the twin without migraine did not have an increased risk of anxious depression. Similar results were obtained when the analysis was restricted to female subjects only (results not shown). Males were not analyzed separately, due to the relatively low number of male discordant twin pairs.

These findings are consistent with an earlier study by Merikangas et al. (1993), who reported that rates of anxiety/depression in relatives of migraineurs were only elevated in the presence of migraine in the relatives. Interestingly, a similar risk pattern can be observed in a series of prevalence diagrams published by Schur and colleagues (2009), which showed that the co-twins of individuals with 'pure' depression (i.e. depression but not migraine) were not at increased risk of 'pure' migraine, and vice versa. Further support for causality comes from a model proposed by de Moor et al. (2008), who argued that if a relationship is causal, all factors influencing the first trait should also affect the second trait. This was indeed the case in our study: genetic and non-shared environmental factors each explained roughly half of the variance in both traits, and genetic and non-shared environmental factors each also explained approximately half of the covariance between migraine and anxious depression.

At present we can only speculate what kind of mechanism might explain a causal relationship between migraine and anxious depression. Possible explanations at the psychological level are that frequent severe migraines might cause depressive or anxious symptoms, or that depressed or anxious patients might over-report pain as a result of their mood disorder. Alternatively, there might be a syndromic association between migraine and anxious depression, as previously suggested by Merikangas et al.(1993). This would indeed be consistent with the theory discussed above, that migraine might be part of the spectrum of symptoms associated with depression. If, in a subgroup of patients, comorbid migraine and depression were aspects of the same disorder, this would provide a good explanation for the pattern of risks we observed in the discordant twin pairs.

Limitations

One potential limitation of this study is the relatively limited power to detect the moderation of migraine heritability by depression. The effects of the moderator were small and only significant when dropped all at once. This indicates an overall moderation effect, but a larger sample is needed to determine whether genetic variance decreases, or whether non-shared environmental variance becomes larger in depressed individuals.

A second potential limitation is the fact that this study used broad definitions of migraine and anxious depression, based on self-report. While this limits comparisons to clinical populations, this strategy has some advantages. First, it is generally not feasible to obtain clinical diagnoses in the large numbers of subjects required for these analyses. Second, in population-based

studies, including data from subclinical cases has the potential to increase the power to detect genetic effects, as we have previously shown for migraine (Ligthart et al., 2008; Nyholt et al., 2004). The same may apply to anxious depression. In practice, using an empirical LCA-based migraine classification results in a prevalence comparable to the combined prevalence of IHS migraine and probable migraine (Lantéri-Minet et al., 2005). Thus, in population-based genetic studies there are clear advantages to using broad, questionnaire-based measures, rather than strict clinical diagnoses only.

Conclusions and implications

Our finding that migraine is less heritable in severely depressed individuals has important implications for research, because it suggests that it may be important to treat migraine with and without comorbid anxiety or depression as separate phenotypes in genetic studies. This is especially worth taking into account when individuals are selected for expensive genotyping efforts. A similar conclusion follows from our findings with respect to causality. If migraine and anxious depression are causally related, 'pure' migraine and migraine associated with anxious depression may not have the same etiology, which could cause considerable genetic heterogeneity.

Comorbidity with migraine has been reported for a wide range of psychiatric (Merikangas et al., 1990) and non-psychiatric conditions (Merikangas et al., 1997; Nyholt et al., 2009; Ottman & Lipton, 1994). Whether our findings extend to other traits beside anxious depression requires further investigation.

Finally, it is worth emphasizing the importance of further research into the nature of migraine in depressed patients. A better recognition and understanding of this phenomenon, resulting in more effective treatment and pain relief, could improve the quality of life of many individuals.

REFERENCES

Bair, M. J., Robinson, R. L., Katon, W., & Kroenke, K. (2003). Depression and pain comorbidity: a literature review. Arch Intern Med, 163(20), 2433-2445.

Boomsma, D. I., Beem, A. L., van den Berg, M., Dolan, C. V., Koopmans, J. R., Vink, J. M., et al. (2000). Netherlands twin family study of anxious depression (NETSAD). Twin Res, 3(4), 323-334.

Boomsma, D. I., de Geus, E. J., Vink, J. M., Stubbe, J. H., Distel, M. A., Hottenga, J. J., et al. (2006). Netherlands Twin Register: from twins to twin families. Twin Res Hum Genet, 9(6), 849-857.

Breslau, N., Davis, G. C., & Andreski, P. (1991). Migraine, psychiatric disorders, and suicide attempts: an epidemiologic study of young adults. Psychiatry Res, 37(1), 11-23.

De Moor, M. H., Boomsma, D. I., Stubbe, J. H., Willemsen, G., & de Geus, E. J. (2008). Testing causality in the association between regular exercise and symptoms of anxiety and depression. Arch Gen Psychiatry, 65(8), 897-905.

Distel, M. A., Ligthart, L., Willemsen, G., Nyholt, D. R., Trull, T. J., & Boomsma, D. I. (2007). Personality, Health and Lifestyle in a Questionnaire Family Study: A Comparison Between Highly Cooperative and Less Cooperative Families. Twin Res Hum Genet, 10(2), 348-353.

Frediani, F., & Villani, V. (2007). Migraine and depression. Neurol Sci, 28 (Suppl 2), S161-165.

Headache Classification Committee of the International Headache Society (2004). The International Classification of Headache Disorders: 2nd edition, Cephalalgia (Vol. 24 [Suppl 1], pp. 9-160).

Kendler, K. S., Neale, M. C., MacLean, C. J., Heath, A. C., Eaves, L. J., & Kessler, R. C. (1993). Smoking and major depression. A causal analysis. Arch Gen Psychiatry, 50(1), 36-43.

Lantéri-Minet, M., Valade, D., Geraud, G., Chautard, M. H., & Lucas, C. (2005). Migraine and probable migraine--results of FRAMIG 3, a French nationwide survey carried out according to the 2004 IHS classification. Cephalalgia, 25(12), 1146-1158.

Lazarsfeld, P. F., & Henry, N. W. (1968). Latent structure analysis. New York: Houghton, Mifflin.

Lépine, J. P., & Briley, M. (2004). The epidemiology of pain in depression. Hum Psychopharmacol, 19 Suppl 1, S3-7.

Ligthart, L., Boomsma, D. I., Martin, N. G., Stubbe, J. H., & Nyholt, D. R. (2006). Migraine with aura and migraine without aura are not distinct entities: further evidence from a large Dutch population study. Twin Res Hum Genet, 9(1), 54-63.

Ligthart, L., Nyholt, D. R., Hottenga, J. J., Distel, M. A., Willemsen, G., & Boomsma, D. I. (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, 147B(7), 1186-1195.

McCutcheon, A. L. (1987). Latent class analysis. Beverly Hills: Sage Publications.

Medland, S. E., Neale, M. C., Eaves, L. J., & Neale, B. M. (2009). A note on the parameterization of Purcell's G x E model for ordinal and binary data. Behav Genet, 39(2), 220-229.

Merikangas, K. R., Angst, J., & Isler, H. (1990). Migraine and psychopathology. Results of the Zurich cohort study of young adults. Arch Gen Psychiatry, 47(9), 849-853.

Merikangas, K. R., Fenton, B. T., Cheng, S. H., Stolar, M. J., & Risch, N. (1997). Association between migraine and stroke in a large-scale epidemiological study of the United States. Arch Neurol, 54(4), 362-368.

Merikangas, K. R., Merikangas, J. R., & Angst, J. (1993). Headache syndromes and psychiatric disorders: association and familial transmission. J Psychiatr Res, 27(2), 197-210.

Merikangas, K. R., & Stevens, D. E. (1997). Comorbidity of migraine and psychiatric disorders. Neurol Clin., 15(1), 115-123.

Mulder, E. J., Van Baal, C., Gaist, D., Kallela, M., Kaprio, J., Svensson, D. A., et al. (2003). Genetic and environmental influences on migraine: a twin study across six countries. Twin Res, 6(5), 422-431.

Neale, M. C., Boker, S. M., Xie, G., & Maes, H. H. (2003). Mx: Statistical Modeling (6 ed.). VCU Box 900126, Richmond, VA 23298: Department of psychiatry.

Nyholt, D. R., Gillespie, N. G., Heath, A. C., Merikangas, K. R., Duffy, D. L., & Martin, N. G. (2004). Latent class and genetic analysis does not support migraine with aura and migraine without aura as separate entities. Genet Epidemiol, 26(3), 231-244.

Nyholt, D. R., Gillespie, N. G., Merikangas, K. R., Treloar, S. A., Martin, N. G., & Montgomery, G. W. (2009). Common genetic influences underlie comorbidity of migraine and endometriosis. Genet Epidemiol, 33(2), 105-113.

Nyholt, D. R., Morley, K. I., Ferreira, M. A., Medland, S. E., Boomsma, D. I., Heath, A. C., et al. (2005). Genomewide significant linkage to migrainous headache on chromosome 5q21. Am J Hum Genet, 77(3), 500-512.

Ottman, R., & Lipton, R. B. (1994). Comorbidity of migraine and epilepsy. Neurology, 44(11), 2105-2110.

Penninx, B. W., Beekman, A. T., Smit, J. H., Zitman, F. G., Nolen, W. A., Spinhoven, P., et al. (2008). The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res, 17(3), 121-140.

Schur, E. A., Noonan, C., Buchwald, D., Goldberg, J., & Afari, N. (2009). A Twin Study of Depression and Migraine: Evidence for a Shared Genetic Vulnerability. Headache, 49(10), 1493-1502.

Schwarz, G. (1978). Estimating the dimension of a model. The Annals of Statistics, 6, 461-464.

Stam, A. H., de Vries, B., Janssens, A. C., Vanmolkot, K. R., Aulchenko, Y. S., Henneman, P., et al. (2010). Shared genetic factors in migraine and depression. Evidence from a genetic isolate. Neurology, 74(4), 288-294.

Sullivan, P. F., Neale, M. C., & Kendler, K. S. (2000). Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry, 157(10), 1552-1562.

8

A genome-wide linkage scan provides evidence for both new and previously reported loci influencing common migraine

Ligthart L., Nyholt D.R., Hottenga J.-J., Distel M.A., Willemsen G., & Boomsma D.I. (2008). A genome-wide linkage scan provides evidence for both new and previously reported loci influencing common migraine. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 147B(7),1186-1195.

ABSTRACT

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.

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; Jones et al., 2001; May et al., 1995; Nyholt et al., 1998; Nyholt et al., 2005; Ophoff et al., 1997; 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; Headache Classification Committee of the International Headache Society, 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 8.1). 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 who do not strictly fulfill the IHS migraine diagnosis will lead to a considerable loss of power in genetic studies of migraine.

TABLE 8.1

Diagnostic criteria for migraine without aura, as published by the International Headache Society (2004)	
A.	At least 5 attacks fulfilling criteria B-D
B.	Headache attacks lasting 4-72 hours (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 (eg, 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

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 (Ligthart et al., 2006; Nyholt et al., 2004). LCA has now been applied successfully in several migraine studies (Lea et al., 2005; Ligthart et al., 2006; Nyholt et al., 2004; Nyholt et al., 2005). 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. (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 8.2).

TABLE 8.2

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	≥ 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 hours
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? †	D2	Photo and phonophobia
partial loss of vision, seeing flashes of light or (zigzag) patterns?		Aura

* An attack frequency of 'several times a year' was assumed to be equivalent to '≥ 5 episodes'.
† 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.

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 (Kallela et al., 2001; Ophoff et al., 1994), 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 (Ligthart et al., 2006; Nyholt et al., 2004). 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 & 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 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 8.1) 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 (Meulenbelt et al., 1995; Miller et al., 1988). 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 & 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 & 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 & Cox exponential model (Kong & 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 ≥ 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 ≥ 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 ≥ 2.4) and significant (LOD ≥ 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.

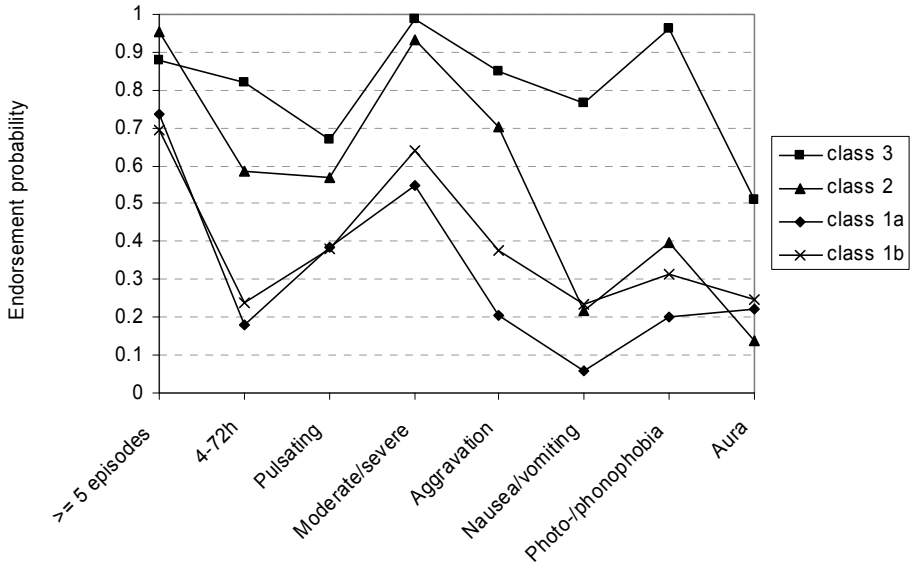

FIGURE 8.1

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.

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 8.1 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 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 8.3 shows the number of individuals with IHS migraine by class membership.

TABLE 8.3

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%)

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 8.2A 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 8.2B.

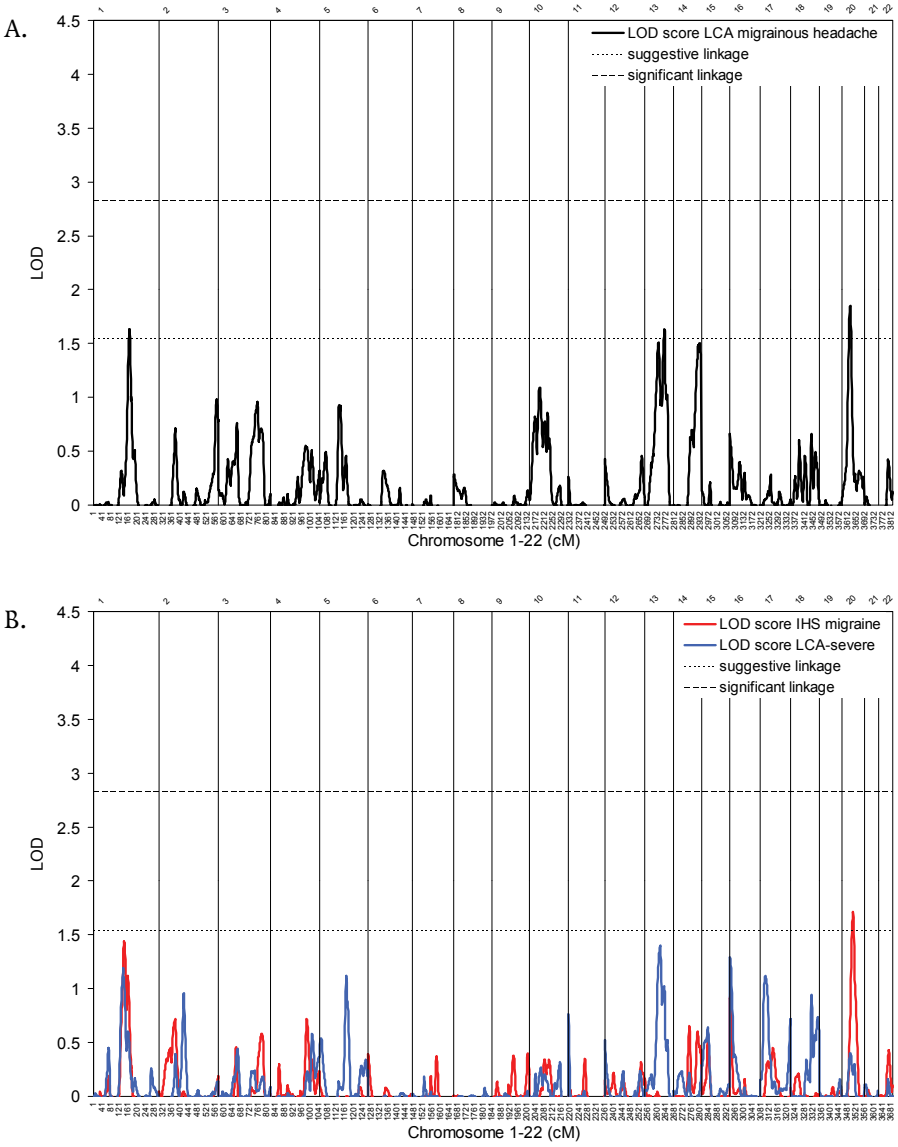

Figure 8.2

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.

The linkage analysis of LCA migrainous headache revealed three LOD scores that exceeded the threshold for suggestive linkage (LOD ≥ 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 (Figure 8.3). Figure 8.3 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 8.4 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 ≥ 2.4) or significant linkage (LOD ≥ 3.85), after correcting for exploratory analysis of multiple phenotypes genome-wide.

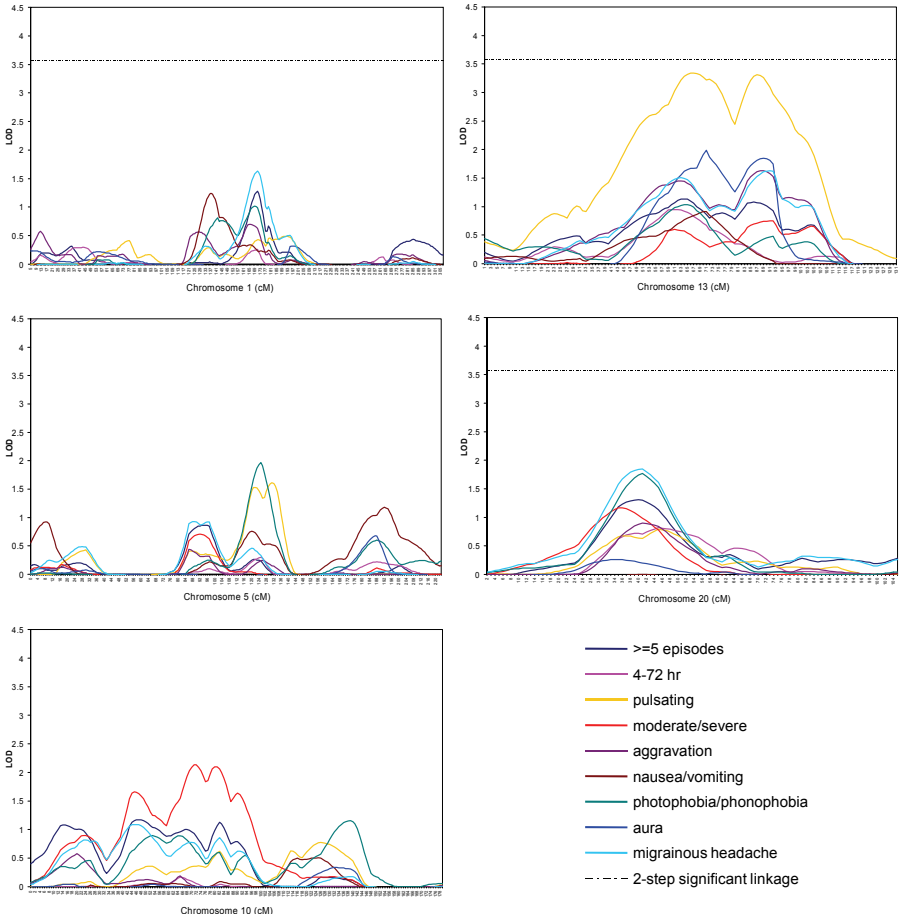

FIGURE 8.3

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.

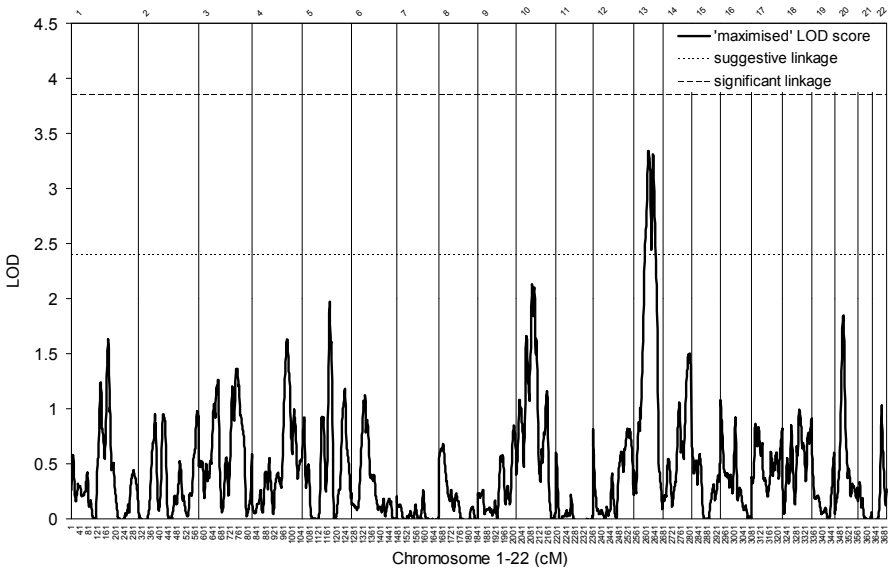

FIGURE 8.4

Chromosome 1-22, genome-wide 'maximised' LOD scores across all nine phenotypes (LCA-derived migrainous headache and individual migraine symptoms).

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 8.4. 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 (Anttila et al., 2006; Nyholt et al., 2005). 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 8.1), 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 (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.

Table 8.4

Main results of genome-wide linkage studies for migraine, and results of the present study at previously reported loci.

Previous Study				Current Study					
Reference	Phenotype	Reference marker	LOD score	Nearest marker	cM from ref. marker	LOD score	Phenotype	N subjects (genotyped) 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
Björnsson et al. (2003)	'loose' MO *	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

* females only; [a] Nonparametric multipoint; [b] Parametric two-point; MO = migraine without aura; MA = migraine with aura.

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 and IHS migraine (Figure 8.2B). 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 8.2 shows that although the locations 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 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 8.3). 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 (Anttila et al., 2006; Nyholt et al., 2005). 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., 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.

REFERENCES

Abecasis, G. R., Cherny, S. S., Cookson, W. O., & Cardon, L. R. (2001). GRR: graphical representation of relationship errors. Bioinformatics, 17(8), 742-743.

Abecasis, G. R., Cherny, S. S., Cookson, W. O., & Cardon, L. R. (2002). Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet, 30(1), 97-101.

Anttila, V., Kallela, M., Oswell, G., Kaunisto, M. A., Nyholt, D. R., Hamalainen, E., et al. (2006). Trait components provide tools to dissect the genetic susceptibility of migraine. Am J Hum Genet, 79(1), 85-99.

Bjornsson, A., Gudmundsson, G., Gudfinnsson, E., Hrafnsdottir, M., Benedikz, J., Skuladottir, S., et al. (2003). Localization of a gene for migraine without aura to chromosome 4q21. Am J Hum Genet, 73(5), 986-993.

Boomsma, D. I., de Geus, E. J., Vink, J. M., Stubbe, J. H., Distel, M. A., Hottenga, J. J., et al. (2006). Netherlands Twin Register: from twins to twin families. Twin Res Hum Genet, 9(6), 849-857.

Cader, Z. M., Noble-Topham, S., Dyment, D. A., Cherny, S. S., Brown, J. D., Rice, G. P., et al. (2003). Significant linkage to migraine with aura on chromosome 11q24. Hum Mol Genet, 12(19), 2511-2517.

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

De Fusco, M., Marconi, R., Silvestri, L., Atorino, L., Rampoldi, L., Morgante, L., et al. (2003). Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. Nat Genet, 33(2), 192-196.

Dichgans, M., Freilinger, T., Eckstein, G., Babini, E., Lorenz-Depiereux, B., Biskup, S., et al. (2005). Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. Lancet, 366(9483), 371-377.

Distel, M. A., Ligthart, L., Willemsen, G., Nyholt, D. R., Trull, T. J., & Boomsma, D. I. (2007). Personality, Health and Lifestyle in a Questionnaire Family Study: A Comparison Between Highly Cooperative and Less Cooperative Families. Twin Res Hum Genet, 10(2), 348-353.

Duffy, D. L. (2006). An integrated genetic map for linkage analysis. Behav Genet, 36(1), 4-6.

Granella, F., D'Alessandro, R., Manzoni, G. C., Cerbo, R., Colucci D'Amato, C., Pini, L. A., et al. (1994). International Headache Society classification: interobserver reliability in the diagnosis of primary headaches. Cephalalgia, 14(1), 16-20.

Headache Classification Committee of the International Headache Society (2004). The International Classification of Headache Disorders: 2nd edition, Cephalalgia (Vol. 24 [Suppl 1], pp. 9-160).

Hovatta, I., Kallela, M., Farkkila, M., & Peltonen, L. (1994). Familial migraine: exclusion of the susceptibility gene from the reported locus of familial hemiplegic migraine on 19p. Genomics, 23(3), 707-709.

Jones, K. W., Ehm, M. G., Pericak-Vance, M. A., Haines, J. L., Boyd, P. R., & Peroutka, S. J. (2001). Migraine with aura susceptibility locus on chromosome 19p13 is distinct from the familial hemiplegic migraine locus. Genomics, 78(3), 150-154.

Joutel, A., Bousser, M. G., Biousse, V., Labauge, P., Chabriat, H., Nibbio, A., et al. (1993). A gene for familial hemiplegic migraine maps to chromosome 19. Nat Genet, 5(1), 40-45.

Kallela, M., Wessman, M., Havanka, H., Palotie, A., & Farkkila, M. (2001). Familial migraine with and without aura: clinical characteristics and co-occurrence. Eur J Neurol, 8(5), 441-449.

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

Lander, E., & Kruglyak, L. (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet, 11(3), 241-247.

Lantéri-Minet, M., Valade, D., Geraud, G., Chautard, M. H., & Lucas, C. (2005). Migraine and probable migraine--results of FRAMIG 3, a French nationwide survey carried out according to the 2004 IHS classification. Cephalalgia, 25(12), 1146-1158.

Lazarsfeld, P. F., & Henry, N. W. (1968). Latent structure analysis. New York: Houghton, Mifflin.

Lea, R. A., Nyholt, D. R., Curtain, R. P., Ovcaric, M., Sciascia, R., Bellis, C., et al. (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, D. I., Martin, N. G., Stubbe, J. H., & Nyholt, D. R. (2006). Migraine with aura and migraine without aura are not distinct entities: further evidence from a large Dutch population study. Twin Res Hum Genet, 9(1), 54-63.

May, A., Ophoff, R. A., Terwindt, G. M., Urban, C., van Eijk, R., Haan, J., et al. (1995). Familial hemiplegic migraine locus on 19p13 is involved in the common forms of migraine with and without aura. Hum Genet, 96(5), 604-608.

McCutcheon, A. L. (1987). Latent class analysis. Beverly Hills: Sage Publications.

Meulenbelt, I., Droog, S., Trommelen, G. J., Boomsma, D. I., & Slagboom, P. E. (1995). High-yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations. Am J Hum Genet, 57(5), 1252-1254.

Miller, S. A., Dykes, D. D., & Polesky, H. F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res, 16(3), 1215.

Neale, M. C., Boker, S. M., Xie, G., & Maes, H. H. (2003). Mx: Statistical Modeling (6 ed.). VCU Box 900126, Richmond, VA 23298: Department of psychiatry.

Nyholt, D. R., Gillespie, N. G., Heath, A. C., Merikangas, K. R., Duffy, D. L., & Martin, N. G. (2004). Latent class and genetic analysis does not support migraine with aura and migraine without aura as separate entities. Genet Epidemiol, 26(3), 231-244.

Nyholt, D. R., Lea, R. A., Goadsby, P. J., Brimage, P. J., & Griffiths, L. R. (1998). Familial typical migraine: linkage to chromosome 19p13 and evidence for genetic heterogeneity. Neurology, 50(5), 1428-1432.

Nyholt, D. R., Morley, K. I., Ferreira, M. A., Medland, S. E., Boomsma, D. I., Heath, A. C., et al. (2005). Genomewide significant linkage to migrainous headache on chromosome 5q21. Am J Hum Genet, 77(3), 500-512.

Ophoff, R. A., Terwindt, G. M., Vergouwe, M. N., Frants, R. R., & Ferrari, M. D. (1997). Wolff Award 1997. Involvement of a Ca2+ channel gene in familial hemiplegic migraine and migraine with and without aura. Dutch Migraine Genetics Research Group. Headache, 37(8), 479-485.

Ophoff, R. A., Terwindt, G. M., Vergouwe, M. N., van Eijk, R., Oefner, P. J., Hoffman, S. M., et al. (1996). Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell, 87(3), 543-552.

Ophoff, R. A., van Eijk, R., Sandkuijl, L. A., Terwindt, G. M., Grubben, C. P., Haan, J., et al. (1994). Genetic heterogeneity of familial hemiplegic migraine. Genomics, 22(1), 21-26.

Soragna, D., Vettori, A., Carraro, G., Marchioni, E., Vazza, G., Bellini, S., et al. (2003). A locus for migraine without aura maps on chromosome 14q21.2-q22.3. Am J Hum Genet, 72(1), 161-167.

Suarez, B. K., & Hodge, S. E. (1979). A simple method to detect linkage for rare recessive diseases: an application to juvenile diabetes. Clin Genet, 15(2), 126-136.

Sullivan, P. F., Montgomery, G. W., Hottenga, J. J., Wray, N. R., Boomsma, D. I., & Martin, N. G. (2006). Empirical evaluation of the genetic similarity of samples from twin registries in Australia and the Netherlands using 359 STRP markers. Twin Res Hum Genet, 9(4), 600-602.

Terwindt, G. M., Ophoff, R. A., van Eijk, R., Vergouwe, M. N., Haan, J., Frants, R. R., et al. (2001). Involvement of the CACNA1A gene containing region on 19p13 in migraine with and without aura. Neurology, 56(8), 1028-1032.

Todt, U., Dichgans, M., Jurkat-Rott, K., Heinze, A., Zifarelli, G., Koenderink, J. B., et al. (2005). Rare missense variants in ATP1A2 in families with clustering of common forms of migraine. Hum Mutat, 26(4), 315-321.

Van den Maagdenberg, A. M., Haan, J., Terwindt, G. M., & Ferrari, M. D. (2007). Migraine: gene mutations and functional consequences. Curr Opin Neurol, 20(3), 299-305.

Vanmolkot, K. R., Kors, E. E., Hottenga, J. J., Terwindt, G. M., Haan, J., Hoefnagels, W. A., et al. (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), 360-366.

Weeks, D. E., & Lange, K. (1988). The affected-pedigree-member method of linkage analysis. Am J Hum Genet, 42(2), 315-326.

Wessman, M., Kallela, M., Kaunisto, M. A., Marttila, P., Sobel, E., Hartiala, J., et al. (2002). A susceptibility locus for migraine with aura, on chromosome 4q24. Am J Hum Genet, 70(3), 652-662.

Wessman, M., Terwindt, G. M., Kaunisto, M. A., Palotie, A., & Ophoff, R. A. (2007). Migraine: a complex genetic disorder. Lancet Neurol, 6(6), 521-532.

9

Genome-wide association for migraine in six population-based European cohorts

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ABSTRACT

This study describes a meta-analysis of genome-wide association studies for common migraine in six population-based European cohorts, with a total sample size of 10,980 individuals (2446 cases, 8534 controls). The participants came from five Dutch studies (Erasmus Rucphen Family study, N = 1546, Netherlands Study of Depression and Anxiety, N = 1530, Netherlands Twin Registry [2 cohorts; N = 1593 and N = 1094], Rotterdam Study, N = 1998), and one Icelandic Study (the AGES-Reykjavik study, N = 3219). The best result in the meta-analysis was found for SNP rs9908234, which had a p-value of 8.00×10^{-8}. The SNP was located in the nerve growth factor receptor *(NGFR)* gene. *NGFR* is a strong candidate for migraine due to its involvement in the trigeminal pain system. Comparison of the meta-analysis results with previously identified candidate genes and linkage regions revealed associations with *ATP1A2*, known to be involved in familial hemiplegic migraine (best SNP rs2854248, $P = 3.62 \times 10^{-4}$), and *GRID2* (best SNP rs1972860, $P = 6.02 \times 10^{-5}$). *GRID2* is a glutamate receptor gene, located on chromosome 4q22, a region which has been reported in several independent linkage studies. These findings indicate that combining the results of population-based GWA studies of migraine is an effective approach for detecting migraine susceptibility genes.

Introduction

Gene-finding studies on migraine to date include primarily linkage studies and candidate-gene association studies. Mutations in three different genes, *ATP1A2* (De Fusco et al., 2003), *SCN1A* (Dichgans et al., 2005), and *CACNA1A* (Ophoff et al., 1996), were found to be responsible for familial hemiplegic migraine (FHM), a rare and severe subtype of migraine that follows a mendelian pattern of inheritance. Finding the genes involved in common migraine has proven more challenging. Although many inconsistent linkage results have been reported for common migraine, some consistently replicated loci are starting to emerge, for instance on chromosome 4q21-q24 (Anttila et al., 2006; Bjornsson et al., 2003; Oedegaard et al., 2009; Wessman et al., 2002) and chromosome 10q22 (Anttila et al., 2006; Anttila et al., 2008; Nyholt et al., 2005). The results of candidate-gene association studies also tend to be inconsistent. The association between migraine and the *MTHFR* gene has been replicated several times (Kara et al., 2003; Kowa et al., 2000; Lea et al., 2004; Rubino et al., 2009; Scher et al., 2006; Schurks et al., 2008), but many other associations have remained single reports (see Colson et al., 2007; de Vries et al., 2009).

These days, genome-wide association has become the method of choice in gene-finding research. Due to the complex nature of common migraine, it is expected that the effects of individual genes will be small, and large samples are needed for sufficient power to detect genetic effects (Visscher, 2008). Here, we present a meta-analysis for common migraine by the DICE consortium. DICE consists of six population-based Dutch and Icelandic samples. Four samples consisted of unrelated subjects from the Netherlands [the Rotterdam Study (Hofman et al., 2007), two cohorts from the Netherlands Twin Registry (NTR; Boomsma et al., 2006), and the Netherlands Study of Depression and Anxiety (Penninx et al., 2008)]. One sample was recruited from a genetically isolated Dutch population (the Erasmus Ruchphen Family study [ERF]; Sleegers et al., 2007; Stam et al., 2010), and one consisted of unrelated individuals from Reykjavik, Iceland (the AGES-Reykjavik study [AGES-RS]; Harris et al., 2007).

Table 9.1

Sample descriptives						
	AGES-RS	**ERF**	**NESDA**	**NTR1**	**NTR2**	**Rotterdam**
Subjects						
Total N:	3219	1546	1530	1593	1094	1998
N cases (♂, ♀):	357 (71, 286)	330 (81, 249)	756 (165, 591)	378 (69, 309	276 (59, 217)	349 (79,270)
N controls (♂, ♀):	2862 (1281,1581)	1216 (615, 601)	774 (322, 452)	1215 (509, 706)	818 (396, 422)	1649 (805,844)
Mean age & SD:	51.22 (±6.33)	48.4 (±14.6)	42.9 (±12.5)	44.8 (±15.0)	48.6 (±14.4)	55.37 (±4.51)
Genotyping & Imputation						
Platform:	Illumina 370CNV	Illumina HumanHap300 HumanHap370 Affymetrix 250K Nsp array	Perlegen/ Affymetrix 600K	Perlegen/ Affymetrix 600K	Illumina Human660W-Quad BeadChip	Illumina Infinium II HumanHap550 version 3.0
Software used:	MACH 1.0.16	MACH	IMPUTE	IMPUTE	IMPUTE	MACH 1.0.15
Reference set:	HapMap CEU	HapMap CEU	HapMap CEU	HapMap CEU	HapMap CEU	HapMap CEU
NCBI build:	36	36	36	36	36	36
HapMap release:	22	22	22	22	24	22
# snps analyzed:	2,408,991	2,135,034	2,432,125	2,431,993	2,542,087	2,450,030
Software for analysis imputed data:	ProbABEL	ProbABEL	SNPTEST	SNPTEST	SNPTEST	ProbABEL

Methods

Subjects

The total sample size in this study was 10,890 individuals (3,219 from AGES-RS; 1,546 from ERF; 1,998 from the Rotterdam Study; 1,530 from NESDA; 1,593 from NTR1 and 1,094 from NTR2). The sample included 2446 migraine cases and 8534 controls. An overview of the samples, including details on sample size and genotyping is provided in Table 9.1.

AGES-RS

The Reykjavik Study is a population-based cohort study established in 1967 to prospectively study cardiovascular disease in Iceland. The cohort included a random sample of men and women born between 1907 and 1935 and living in Reykjavik at baseline. In 2002, the Reykjavik Study continued as the AGES-Reykjavik Study to examine risk factors, genetic susceptibility, and gene-environment interactions in relation to disease and disability in old age. Headache data were collected as part of the Reykjavik study. The Reykjavik Study and AGES-Reykjavik Study have been described in detail elsewhere (Harris et al., 2007; Jonsdottir et al., 2002; Scher et al., 2009; Sigurdsson et al., 1995). The AGES-Reykjavik study included 357 migraine cases (71 male, 286 female). The control group included 1281 males and 1581 female, a total of 2862 controls. The mean age was 51.03 years (SD = 6.37). All subjects were unrelated.

ERF

The ERF study is a family-based study in a genetically isolated population in the southwest of the Netherlands. This young genetic isolate was founded in the mid 18th century and minimal immigration and/or marriages occurred between surrounding settlements due to social and religious reasons. The ERF population includes 3,465 individuals that are living descendants of 22 couples with at least six children baptized in the community church around 1850–1900. The subjects were unselected with respect to phenotypes. Details about the extensive genealogy and pedigree of the population are described elsewhere (Santos et al., 2006).

The present study includes data from 1546 ERF participants; 330 migraineurs and 1216 controls. Of the cases, 81 (25%) were male and 249 (75%) were female; of the controls, 615 (51%) were male and 601 (49%) were female. The mean age was 48.4 years (SD = 14.6).

NESDA

The NESDA cohort consisted of 1530 unrelated individuals from the Netherlands (mostly MDD patients) who were genotyped in the context of the GAIN MDD study. In the NESDA sample, 1383 subjects had MDD, and 147 were selected for low risk of MDD. In this sample, there were 756 individuals with migraine (713 with MDD and 43 without MDD) and 774 controls (670 with MDD and 104 without MDD). In the case group, 165 individuals (22%) were male and 591 (78%) were female. In the control group, 322 (42%) were male and 452 (58%) were female. The mean age was 42.9 years (SD = 12.5).

NTR1

The Netherlands Twin Registry collects data in Dutch twins, their parents, siblings and partners. The migraine data were collected in the context of a longitudinal study on health, lifestyle and personality. The first NTR cohort was genotyped as part of the NIH GAIN project, for a GWAS study originally designed to find genes for major depressive disorder (MDD; Boomsma et al., 2008). The majority of subjects (N = 1481) were selected for low risk of MDD, 112 subjects were MDD patients. Migraine data were available for 1593 individuals: 378 cases [56 with MDD and 322 without MDD], and 1215 controls [56 with MDD and 1159 without MDD]. In the case group, 69 individuals (18%) were male and 309 (82%) were female. In the control group, 509 (42%) were male and 706 (58%) were female. The mean age was 44.8 years (SD = 15.0). All subjects were unrelated.

NTR2

The second cohort from the Netherlands Twin Registry was an unselected sample. All subjects were unrelated. For 1094 individuals, migraine data were available. There were 276 migraine cases, including 59 (21%) males and 217 (79%) females. The control group consisted of 818 controls, including 396 (48%) males and 422 (52%) females. The mean age in this cohort was 48.6 years (SD = 14.4).

Rotterdam Study

This sample included participants of the Rotterdam Study, a prospective population based cohort study among persons 55 years or older who were living in Ommoord, a well-defined district of Rotterdam, the Netherlands (Hofman et al., 2007). The aim of this study was to investigate causes of frequent chronic diseases, with a focus on cardiovascular, neurologic, psychiatric, and

ophthalmic diseases. The Medical Ethics Committee of Erasmus Medical Center approved of the study. The original cohort of the Rotterdam Study (7,983 participants) was expanded in 2000 (N = 3,011) and again in 2006 to include 3,919 persons who were 45 years of age or older. At study entry all participants underwent a structural interview and a physical examination, which was repeated every 3-4 years. The migraine questionnaire was introduced into the core study protocol in 2006 (response rate of 64.8%). For the current report, we used data from persons from the second cohort expansion (2006 to 2008) who completed the migraine questionnaire.

Migraine data were available for 1,998 unrelated individuals, including 349 cases (79 male, 270 female) and 1,649 controls (805 male, 844 female). The mean age of the sample was 55.37 years (SD=4.51).

Phenotypes

AGES-RS

Subjects reporting headache at least once a month were asked whether the headaches were accompanied by any of the following migraine features: nausea/vomiting, unilateral location, photophobia, visual disturbance during or preceding headache, and unilateral numbness preceding headache. Individuals were defined as having migraine with aura if they had visual or sensory aura, or both. Subjects with at least 2 of the non-aura symptoms were classified as having migraine without aura. In this study, both migraine with and without aura were included as cases. The remaining individuals were included as controls.

ERF

Migraine was diagnosed according to ICHD-II criteria (Headache Classification Committee of the International Headache Society, 2004). Migraineurs were identified using a three-stage screening procedure which has been validated in a population based study (Launer et al., 1999). The screening procedure is described in detail by Stam et al. (2010). In brief, all participants filled out a concise screening questionnaire on headache and aura symptoms, and those who screened positive also completed a detailed questionnaire. All participants who screened positive were telephone-interviewed to clarify their clinical symptoms. Final diagnosis was always made after this telephone interview and in consultation with a neurologist specialized in headache. The control group consisted of ERF participants negative for migraine based on the written questionnaire.

NESDA, NTR1, NTR2

Migraine was assessed with a questionnaire that provided information on the symptoms listed in the ICHD-II criteria. For the NTR participants, the headache questions were embedded in surveys that were held in the context of a longitudinal study on health, lifestyle and personality. The data used in this study were collected in two waves that took place in 2002 and 2004. Both surveys included the same set of headache items. Data collection procedures are described in detail elsewhere (Boomsma et al., 2006; Distel et al., 2007). When a participant answered the headache section in both surveys, the most recent (2004) survey was used.

The NESDA participants underwent a 4-hour baseline assessment at one of seven clinic sites at the beginning of the study. This assessment included an interview on somatic health, functioning and health care use, and the administration of several written questionnaires. Headache data were collected using the using the same questionnaire that was included in the NTR survey. Further details on the NESDA data collection procedures can be found elsewhere (Penninx et al., 2008).

Individuals screening positive for a screening question ('Do you ever experience headache attacks, for instance migraine?') subsequently answered a set of more detailed questions about their headaches. This information was used to determine the presence of eight of the symptoms present in the ICHD-II criteria: moderate/severe pain intensity, aggravation by physical activity, pulsating quality, nausea/vomiting, photo-/phonophobia. The IHS migraine symptom variables were analysed with Latent Class Analysis to determine each participant's affection status for migrainous headache. This method has been described extensively in previous studies . The LCA was performed based on headache data from all available NESDA and NTR participants, using the program Latent Gold 4.0 (Statistical Innovations, Inc., Belmont, MA).

Rotterdam Study

The migraine questionnaire was based on the ICHD-II criteria and was a modified questionnaire according to the GEM study of Leiden (Launer et al., 1999). The first question was "Have you ever experienced a severe headache that affected your daily activities?" If the answer was negative or if it was clearly indicated that the participants experienced a severe headache due to other causes, such as a tumor, sinusitis, stroke, trauma or meningitis, no further questions on headaches were asked. If the answer to the first question was positive, headache duration and headache frequency were asked. Next, if a

person experienced headaches of which 1) the duration was between 4 and 72 hours (untreated) or the participant did not know the answer to this question, because they always treated their headache attack and 2) the attack frequency was two or more attacks in a lifetime, details on the characteristics and symptoms of the headaches were asked. These included age of onset, unilateral location, pulsating quality, aggravation by daily activities, sensitivity to light and sound, nausea or vomiting. The frequency of the symptoms accompanying the headaches was assessed and defined as never, sometimes, half of the time and more than half of the time. In this group of participants, questions on medication use were assessed. Furthermore, every participant was asked about aura symptoms and physician diagnosis, if they ever had a severe headache. If the participant experienced an aura or the physician had diagnosed migraine, questions on medication use were assessed. Participants whose duration of headache was unknown, because they always used medication to prevent or treat the attack, were considered migraineurs if they fulfilled the remaining IHS criteria. Individuals who were not classified as migraineurs were included as controls.

Genotyping and imputation

AGES-RS

Genotyping was performed using the Illumina 370CNV platform. Genotypes for ~2.5 million SNPs were imputed using the MACH 1.0.16 program, using HapMap CEU as the reference set, based on NCBI build 36, HapMap release 22.

ERF

Genotyping was performed on several different platforms (Illumina HumanHap300, HumanHap370, Affymetrix 250K Nsp array). These sets were merged and genotypes for ~2.5 million SNPs were imputed to HapMap CEU, release 22, NCBI build 36 using the MACH program. Data were filtered for rare variants and LD (MAF < 0.05 were excluded, SNPs with an R^2 below 0.3 were excluded). The study-specific genomic inflation factor (λ) of 1.166, reflecting relatedness between study participants, was corrected for by applying genomic control.

NTR1 and NESDA

Individual genotyping for the GAIN sample was conducted by Perlegen Sciences (Mountain View, CA, USA) using a set of four proprietary, high-density oligonucleotide arrays. The SNPs on these arrays were selected to tag common

variation in the HapMap European and Asian panels. Of the 3820 Dutch samples sent to Perlegen, genotypes were delivered for 3761 samples. After quality control, there were 3540 subjects in the final analysis dataset (1738 MDD cases and 1802 controls).

The unfiltered dataset obtained from dbGaP contained 599,156 unique SNPs. To be included in the final analysis dataset, SNPs were required not to have any of the following features: gross mapping problem, >= 2 genotype disagreements in 40 duplicated samples, >= 2 Mendelian inheritance errors in 38 complete trio samples, minor allele frequency < 0.01, or > 0.05 missing genotypes in either cases or controls. A total of 427,049 autosomal SNPs met these criteria and were included in the analyses.

Genotypes for ~2.5 million SNPs were imputed using the IMPUTE software, using the HapMap CEU data (release 22, NCBI build 36), available from the IMPUTE website (https://mathgen.stats.ox.ac.uk/impute/impute.-html), as reference. For each SNP an R^2 value was calculated using the QUICKTEST program (http://toby.freeshell.org/software/quicktest.shtml). SNPs were excluded if the HWE test in controls produced a p-value < 1×10^{-6}, the minor allele frequency (MAF) was smaller than 1%, and the R^2 was smaller than 0.3, leaving 2,432,125 SNPs for analysis in the NESDA sample and 2,431,994 in the NTR sample.

NTR2

Genotyping for 657,366 was performed SNPs on the Human660W-Quad BeadChip. SNPs were excluded based on MAF < 0.01, missing genotype rate > 0.05 or a p-value < 1×10^{-5} in a test of Hardy-Weinberg equilibrium. After quality control, 515,781 SNPs were left. Genotypes of ~3.8 million SNPS were imputed with the IMPUTE program (Marchini et al., 2007), using the HapMap CEU data (release 24, NCBI build 36), available from the IMPUTE website, as reference. Imputed SNPs were excluded if they had a minor allele frequency < 0.01 or an R^2 < 0.3, leaving 2,506,433 SNPs for analysis.

Rotterdam Study

Genotyping was performed using the Illumina Infinium II HumanHap550 chip, version 3.0. A total of 572,129 SNPs were genotyped. SNPs were excluded based on the following criteria: HWE p-value < 1×10^{-6}, call rate < 98% and a minor allele frequency < 0.01. The number of SNPs that survived quality control was 514,139. Genotypes were imputed for 2,543,888 SNPs, using the Hapmap CEU (build 36, rel. 22) as reference. Imputations were performed in MACH 1.0.15.

SNPs were excluded if they had a minor allele frequency < 0.01 or an R^2 < 0.3, leaving a total of 2,450,030 SNPs for analysis.

Meta-analysis

In each sample, a logistic regression association test was performed, with sex, age, and age^2 included as covariates, under an additive model. Age^2 was included to account for potential nonlinearity of the age effect, because the prevalence of migraine is lower in both younger and older individuals (Stewart et al., 1992). Uncertainty of imputation was taken into account in the analyses. The data of AGES-RS, ERF and Rotterdam were analyzed with ProbABEL (Aulchenko et al., 2007), NESDA, NTR1 and NTR2 were analysed using SNPTEST (Marchini et al., 2007). The study specific genomic inflation factors (λ) were 1.002, 1.000, 1.006, 1.013, 1.000 and 1.021 for AGES-RS, ERF, NESDA, NTR1, NTR2 and Rotterdam, respectively.

Next, a meta-analysis was performed in the six samples (total N = 10,890) with the METAL program (http://www.sph.umich.edu/csg/abecasis/metal/). Since different phenotype definitions were used in the different samples, the effect sizes were not directly comparable between studies. Therefore, a pooled *Z*-score approach was used. With the pooled *Z*-score method, an overall *Z*-score is calculated based on the summed *Z*-scores from the individual studies, weighted by each study's sample size. The weights are calculated as the square root of (N_{study}/N_{total}). The squared weights sum to one. The sign of the *Z*-score indicates the direction of effect. To ensure that meta-analysis results were indeed based on a substantial number of samples, SNPs present for less than 70% of all participants (N = 184,350) were excluded from the meta-analysis. This left a total of 2,394,913 autosomal SNPs for analysis.

Comparison of results with evidence from previous studies

The outcomes of the meta-analysis were compared to previously reported candidate genes and linkage regions for migraine. Candidate genes were selected from the literature, and were included if at least one study reported a significant association with migraine for this gene. In addition, the three genes indentified for FHM (*ATP1A2*, *SCN1A* and *CACNA1A*) were assessed.

Genome-wide linkage studies for migraine were identified with a literature search in PubMed. A selection was made of SNPs with a p-value < 1 x 10^{-4} in the meta-analysis. In regions where multiple SNPs had small p-values, the best SNP was selected. The locations of the best SNPs were compared to the locations of the best markers in previously reported linkage regions. Since not

all studies clearly specified the confidence intervals of their linkage peaks, results were included if the distance between the best linkage marker and the SNP was less than 15 Mb.

RESULTS

In Figure 9.1, the Q-Q plot for the meta-analysis is shown. The genomic inflation factor λ was 1.022. Figure 9.2 shows the Manhattan plot for this analysis. Table 9.2 shows an overview of the meta-analysis results, for all SNPs with a p-value of 1×10^{-5} or smaller. In regions where multiple SNPs had p-values $< 1 \times 10^{-5}$, the SNP with the best p-value is reported, and the number of SNPs with small p-values surrounding the best SNP is given.

The most significant result was obtained in SNP rs9908234 ($P = 8.00 \times 10^{-8}$), located in the nerve growth factor receptor *NGFR*. A total of 17 SNPs were tested in this gene; the other SNPs were not associated with migraine, and not in LD with rs9908234 (Figure 9.3). The SNP was genotyped in the NTR1 and NESDA samples, and imputed in the other samples. Table 9.3 lists the results for the individual samples.

The top results were compared with the literature using the text-mining program Anni (version 2.1, http://www.biosemantics.org/index.php?page=-anni-2-0). Of all genes associated with SNPs that had p-values $< 1 \times 10^{-4}$, *NGFR* was identified as the best candidate based on literature connections with the concept 'migraine'. Table 9.4A shows the results of the meta-analysis in candidate genes for migraine. A series of 22 SNPs with p-values < .05 was identified in estrogen receptor 1 *(ESR1)*. This is also a large gene (~412 Kb), and the meta-analysis included 360 SNPs within this gene. The nominally significant SNPs clustered within a region of ~90 Kb. Also, two series of nominally significant SNPs were found in the progesterone receptor *(PGR)* and the 5-hydroxytryptamine (serotonin) receptor 2A *(HTR2A)*. Table 9.4B shows the results of the meta-analysis in the three known FHM genes. The best results in the meta-analysis were obtained for *ATP1A2*. In this gene, 20 SNPs were tested, 3 of which had a p-value < .001. The best result was found for rs2854248 ($P = 3.62 \times 10^{-4}$). Good results were also obtained in the *CACNA1A* gene. This is a large gene (spanning about 300Kb), and within this gene 241 SNPs were analyzed. 13 of the 17 SNPs with p-values < 0.05 were confined to a small region of ~26 Kb, surrounding the second and third exon. The best SNP was rs3764615 (P = .004).

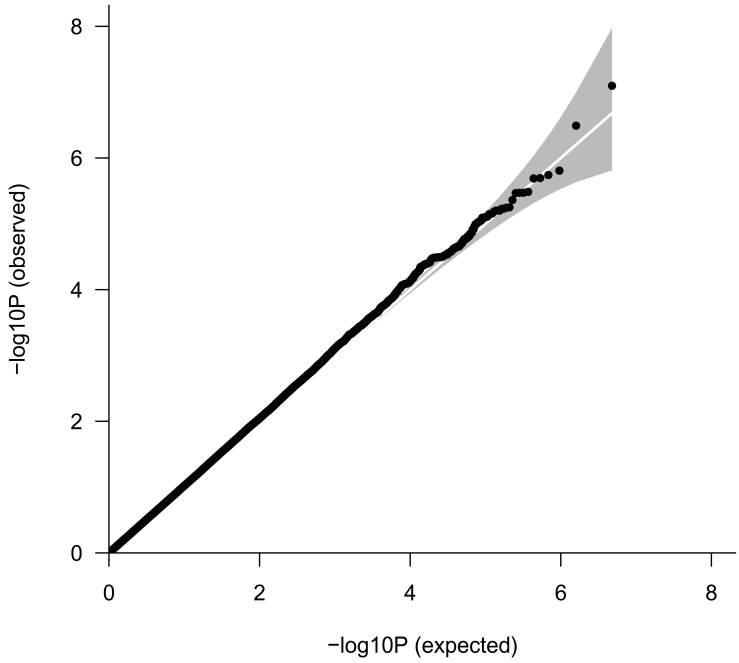

Figure 9.1

Q-Q plot showing the expected and observed distribution of p-values in the meta-analysis. The genomic inflation factor (λ) for the meta-analysis including all six samples was 1.022.

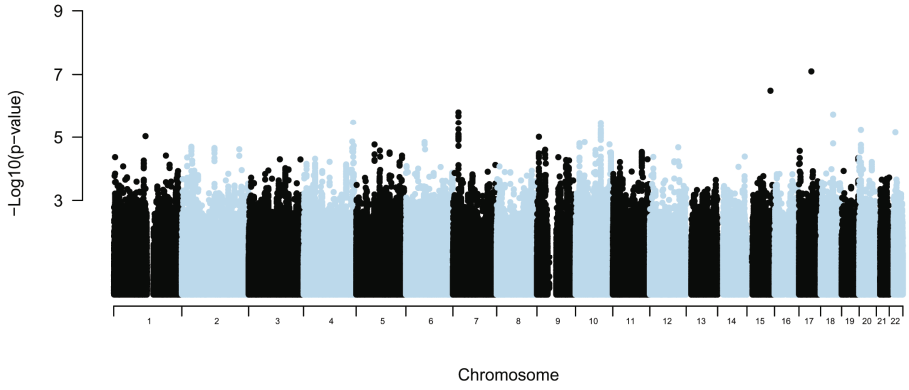

Figure 9.2

Manhattan plot showing the results of the meta-analysis.

Table 9.2

Most significant snps per region											
SNP	Chr	Position (bp)	Type	Closest gene	Distance to gene (bp)	A1	A2	Z-score	P-value	Direction of effect	N SNPs in region ($P < 1 \times 10^{-05}$)
rs9908234	17	44932347	intronic	NGFR	0	A	G	-5.367	8.00×10^{-08}	------	1
rs11636768	15	85496515	intergenic	AC020687	321903	A	G	5.109	3.23×10^{-07}	++++?+	1
rs10275320	7	20148579	intronic	MACC1	0	A	G	-4.804	1.56×10^{-06}	------	8
rs4939879	18	45399981	intergenic	LIPG	26705	A	G	4.773	1.82×10^{-06}	++++++	1
rs4861775	4	180553645	intergenic	AC017087.1	-709541	A	C	-4.653	3.28×10^{-06}	------	1
rs986222	10	91920867	intergenic	AL139340.2	-7170	A	G	4.647	3.37×10^{-06}	++++++	16
rs6107848	20	6539116	intergenic	AL121911	82010	A	G	4.530	5.90×10^{-06}	+++++-	1
rs140174	22	22252983	intronic	IGLL1	0	A	G	-4.494	6.98×10^{-06}	------	1
rs1146161	1	115460299	intergenic	AL109660.1	13497	A	C	4.433	9.27×10^{-06}	++++++	1
rs4742323	9	7276743	intergenic	KDM4C	111095	C	G	-4.424	9.70×10^{-06}	------	1

The best SNP per region is shown, as well as the number of SNPs in the region with a p-value < 1×10^{-5}. The 'Direction' column shows the direction of effect of the best SNP in the region, for each of the six samples, in the following order: AGES-RS, ERF, NESDA, NTR1, NTR2, Rotterdam. A question mark indicates the SNP has not been tested for a particular sample, because it was removed during quality control. A1 is the effect allele, A2 is the non-effect allele. Positions are based on NCBI Build 36.

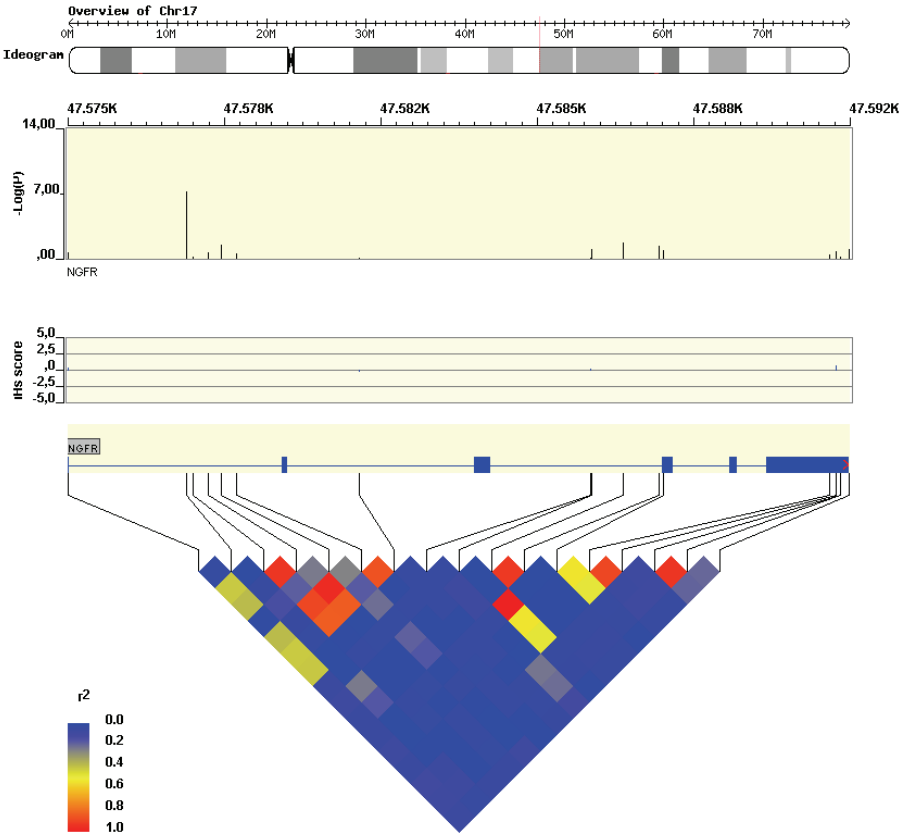

Figure 9.3

Plot showing the LD structure between the SNPs tested in NGFR. Rs9908234 was not in LD with any of the other SNPs tested.

TABLE 9.3

Details on rs9908234 for the individual samples							
	Beta	SE	OR	P-value	Imp	R^2	MAF
AGES-RS	0.763	0.185	2.145 (1.493 - 3.081)	3.6×10^{-05}	yes	0.55	0.06
ERF	0.306	0.134	1.357 (1.043 - 1.767)	0.023	yes	0.61	0.10
NESDA	0.423	0.149	1.527 (1.140 - 2.045)	4.5×10^{-03}	no	-	0.07
NTR1	0.256	0.186	1.292 (0.896 - 1.861)	0.170	no	-	0.06
NTR2	0.215	0.270	1.240 (0.730 - 2.105)	0.426	yes	0.63	0.05
Rotterdam	0.229	0.217	1.257 (0.822 - 1.922)	0.297	yes	0.59	0.07
SE = standard error; OR = odds ratio; Imp = imputed; MAF = minor allele frequency							

In Table 9.5 a comparison is given of the results of the meta-analysis and previous results of genome-wide linkage studies of migraine (published between 2002 and 2010). The locations of SNPs with a p-value < 1×10^{-4} were compared with the locations of the best markers in previously reported linkage regions. Results were included if the distance between the linkage marker and the SNP was less than 15 Mb. Of particular interest is the overlap of the linkage region on chromosome 4q22 and a SNP in the glutamate receptor, ionotropic, delta 2 *(GRID2)* gene (rs1972860, P = 6.02×10^{-5}).

DISCUSSION

This is the first meta-analysis of migraine in population-based cohorts. The analysis was based on data from 10,890 individuals (2446 cases, 8534 controls) of European ancestry. The best result based on all six samples was found for a SNP on chromosome 17, located in the nerve growth factor receptor *(NGFR)* gene. The nerve growth factor receptor, also known as the p75 neurotropin receptor (p75NTR) is part of a large superfamily of tumor necrosis factor receptors. NGFR is one of two receptors that bind neural growth factor (NGF). NGF acts as a peripheral pain mediator, and is upregulated in many chronic pain conditions, particularly in inflamed tissues (Pezet & McMahon, 2006). The tyrosine kinase receptor A (TrkA) is another receptor that binds NGF, and the NGF-NGFR pathway has been reported to play an important role in the transmission of pain.

Specifically, NGF can activate and sensitize primary afferent neurons that express TrkA, producing hyperalgesia (Woolf et al., 1994). The NGF-NGFR pathway has been studied less frequently, but experiments in mice and rats suggest this pathway is also important in pain transmission (Fukui et al., 2010). A study by Zhang & Nicol (2004) suggested an important role of p75NTR in NGF-induced sensitization of sensory neurons in adult rat dorsal root ganglia.

The NGF receptor has not been linked directly to migraine in the literature, however, there is some evidence for the involvement of NGF in chronic headache disorders. Sarchielli et al. (2001) found increased levels of NGF in the cerebrospinal fluid (CSF) of patients with chronic daily headache, whereas Blandini et al. (2006) found reduced peripheral levels of NGF in migraineurs. Based on current knowledge, the hypothesis that NGFR mediates NGF-induced sensitization of trigeminal neurons seems to provide a plausible explanation for migraine headache.

Additional interesting findings concern some of the previously reported candidate genes for migraine. In particular, a strong association was detected with SNPs located in the *ATP1A2* gene. This gene has previously been implicated in FHM (De Fusco et al., 2003). Previous linkage findings in the 1q23 region already supported a possible role of *ATP1A2* in common migraine (Ligthart et al., 2008; Nyholt et al., 2005). In addition, two *ATP1A2* mutations were identified in a study that performed a *ATP1A2* mutation screen in MO and MA families, suggesting involvement of this gene in common migraine (Todt et al., 2005). The present study provides convincing further evidence for this hypothesis.

There was also considerable overlap between potentially interesting SNPs and previously reported linkage findings. Particularly interesting is the finding that the glutamate receptor, ionotropic, delta 2 *(GRID2)* gene on chromosome 4q22 shows a strong association with migraine in this meta-analysis. The 4q22 region has been reported in several independent linkage studies (Anttila et al., 2006; Bjornsson et al., 2003; Oedegaard et al., 2009; Wessman et al., 2002). Evidence for the involvement of glutamate in migraine comes mostly from studies on FHM; the mutations that cause FHM all result in an increase of synaptic glutamate levels (van den Maagdenberg et al., 2007). Other studies have reported elevated levels of glutamate in the CSF of migraine patients (Martinez et al., 1993; Peres et al., 2004). Glutamate receptors have previously been suggested as promising targets for migraine treatment (Andreou & Goadsby, 2009). The results of the present study support this hypothesis.

Table 9.4A

Results of the meta-analysis in previously identified candidate genes													
Gene Symbol	Study*	Location	Best SNP in meta-analysis	BP	Pooled Z-score	Pooled P-value	Direction of effect	A1	A2	#P < .01	# P <.05	# P <.1	Total # SNPs
MTHFR	1-5	1p36.3	rs4846049	11772952	-1.802	0.07153	−+−−−−	T	G	0	0	5	35
HTR2B	7	2q36.3-37.1	rs6437000	231685771	1.682	0.0925	++++−+	A	C	0	0	1	10
EDNRA	8,9	4q31	rs17675063	148657950	2.243	0.02487	+?++++	A	G	0	1	3	47
LTA	10-12	6p21.3	rs3093542	31648672	2.553	0.01069	++++++	C	G	0	1	1	4
TNF	13	6p21.3	rs3093662	31652168	-1.405	0.16	−−−−−−	A	G	0	0	0	3
ESR1	14, 15	6q25.1	rs9322336	152242123	-3.186	0.001445	−−−−−−	T	C	9	23	42	425
DDC	7	7p11	rs2167364	50533321	2.138	0.03252	++++++	T	C	0	5	41	195
STX1A	16	7q11.2	rs941299	72763115	1.395	0.1631	+−−+++	T	C	0	0	0	8
DBH	17-19	9q34	rs129882	135513490	1.841	0.06558	++−+++	T	C	0	0	1	52
GSTP1	20	11q13	rs749174	67109829	2.071	0.03837	+++++−	A	G	0	1	3	5
PGR	21	11q22-q23	rs503362	100467037	-2.62	0.008784	−−−−−−	C	G	1	28	32	137
DRD2	19, 22	11q22-q23	rs4586205	112812339	-1.969	0.04895	−−+−−+	T	G	0	1	7	74
HTR2A	23	13q14-q21	rs7330636	46321593	2.47	0.01352	+−++−+	T	C	0	18	27	113

Table 9.4A (continued)

SLC6A4	24, 25	17q11.2	rs4251417	25575984	-1.743	0.08127	+−−−−−	T	C	0	0	1	26
ACE	26-28	17q23	rs4305	58911961	-1.095	0.2733	−++−−−	A	G	0	0	0	15
INSR	29	19p13.3-13.2	rs8103483	7096374	1.995	0.046	++++−+	T	C	0	1	12	144
LDLR	30	19p13.3	rs1799898	11088554	2.379	0.01734	+−−+++	T	C	0	1	2	38
NOTCH3	31	19p13.2-13.1	rs10426042	15135770	-1.663	0.09624	−−−+−−	C	G	0	0	1	27

The best results in the meta-analysis, located within genes previously reported to be associated with migraine in a candidate-gene study. *Numbers refer to the following studies: 1 Kowa et al., (2000); 2 Kara et al., (2003); 3 Lea et al., (2004); 4 Scher et al., (2006); 5 Rubino et al., (2009); 6 Schurks et al., (2008); 7 Corominas (2010); 8 Tzourio (2001); 9 Tikka-Kleemola (2009); 10 Trabace et al., (2002); 11 Lee et al., (2007); 12 Asuni et al., (2009); 13 Rainero et al., (2004); 14 Colson et al., (2004); 15 Oterino et al., (2006); 16 Corominas et al., (2009); 17 Lea et al., (2000); 18 Fernandez et al., (2009); 19 Todt et al., (2009); 20 Kusumi et al., (2003); 21 Colson et al., (2005); 22 Peroutka et al., (1997); 23 Erdal et al., (2001); 24 Yilmaz et al., (2001); 25 Marziniak et al., (2005); 26 Paterna et al., (1997); 27 Paterna et al., (2000); 28 Kowa et al., (2005); 29 McCarthy et al. (2001); 30 Mochi et al., (2003); 31 Schwaag et al., (2006).

Table 9.4B

Results of the meta-analysis in genes previously implicated in familial hemiplegic migraine												
Gene Symbol	Study*	Location	Best SNP in meta-analysis	BP	Pooled Z-score	Pooled P-value	Direction of effect	# P < .001	# P < .01	# P < .05	# P < .1	Total # SNPs
ATP1A2	1	1q21-q23	rs2854248	158360551	3.566	0.0003618	++++++	3	4	5	8	20
SCN1A	2	2q24.3	rs12151636	166630459	2.142	0.03218	+?+−++	0	0	1	1	99
CACNA1A	3	19p13	rs3764615	13424952	2.903	0.003695	−+++−+	0	9	17	37	241

The best results in one of the genes implicated in FHM. The direction of effect for the best SNP is indicated per sample, in the following order: AGES-RS, ERF, NESDA, NTR1, NTR2, Rotterdam. A question mark indicates the SNP has not been tested for a particular sample, because it was removed during quality control. The number of SNPs with p-values below .001, .01, .05 and .1 are indicated, as well as the total number of SNPs tested within the gene. A1 is the effect allele, A2 is the non-effect allele.

*Numbers refer to the following studies: 1 De Fusco et al., (2003); 2 Dichgans et al., (2005); 3 Ophoff et al., (1996).

Strengths and limitations

A potential limitation of this study is that migraine definitions were not the same across cohorts. Although in all studies the definition of migraine was largely based on the symptoms specified in the ICHD-II diagnostic criteria, there were differences in how the endpoint diagnosis was reached, with some studies using more liberal criteria than others. This makes it difficult to compare effect sizes across studies. For this reason the meta-analysis was not based on effect sizes, but on *Z*-scores. The beta values, representing the effect size, were only used to determine the direction of the effect. The disadvantage of this method is that a direct comparison of the effect sizes in individual studies is not possible. However, by using a *Z*-score-based approach we avoid drawing conclusions based on comparing effect sizes that are not on the same scale.

The most significant p-value in this study just failed to reach genome-wide significance [commonly defined by a threshold of 5×10^{-8} (e.g., Dudbridge & Gusnanto, 2008)]. There are several reasons why we do not expect to find exceptionally strong association signals in this type of study. First, migraine is a complex disorder, and the effects of individual genes are expected to be small. The fact that FHM, which follows a mendelian pattern of inheritance, can be caused by dozens of different mutations in at least 3 different genes (de Vries et al., 2009), indicates it is not unlikely that the more complex forms of migraine are probably even more heterogeneous in nature. Second, this study is population-based, and subjects were unselected with respect to migraine status. For this reason it is important to include not only the severest cases, but also more mildly affected patients, to capture the maximum amount of genetic information on migraine. However, because there was no selection with respect to phenotype, the number of cases is relatively small compared to the number of controls, and cases are expected to be less severely affected than cases in a study sample specifically selected for migraine, for instance in a clinical study.

Table 9.5

Comparison of meta-analysis with regions reported in genome-wide linkage studies of migraine

Closest SNP	Location	P-value SNP	Closest gene	Type	Linkage study*	Phenotype	Best marker	Location marker	Dist. to SNP (Mb)	LOD score
rs1972860	4q22.2	6.02×10^{-05}	GRID2	intronic	1	age at onset	D4S2380	4q22	1.30	HLOD 1.96[c]
rs1972860	4q22.2	6.02×10^{-05}	GRID2	intronic	2	MO	D4S1534	4q21.23	8.27	2.05[b]
rs1972860	4q22.2	6.02×10^{-05}	GRID2	intronic	1, 3, 4	MA, various, migraine broad	D4S1647	4q23	4.86	4.20[c], HLOD 4.52[c]†, 2.26[b]
rs2303655	5q23.2	3.05×10^{-05}	ZNF474	intergenic	5, 6	LCA, photo/phono	D5S2501	5q22.1	11.48	3.70[b]†, 1.97[b]
rs6919479	6p21.1	9.86×10^{-05}	CDC5L	intergenic	7	MO and MA	D6S452	6p12.2-21.1	2.46	5.41[c]
rs10999688	10q22.1	2.55×10^{-05}	UNC5B	intergenic	5	LCA migraine	D10S2327	10q22.3	7.83	2.32[b]
rs10999688	10q22.1	2.55×10^{-05}	UNC5B	intergenic	8	Various phenotypes	D10S1786	10q23.1	11.37	7.68[b]†
rs11200686	10q23.1	5.76×10^{-05}	AL390786.1	intergenic	5	LCA migraine	D10S2327	10q22.3	5.21	2.32[b]
rs11200686	10q23.1	5.76×10^{-05}	AL390786.1	intergenic	8	Various phenotypes	D10S1786	10q23.1	1.67	7.68[b]†
rs3862561	10q23.1	5.65×10^{-05}	AC069540.4	intergenic	5	LCA migraine	D10S2327	10q22.3	5.19	2.32[b]
rs3862561	10q23.1	5.65×10^{-05}	AC069540.4	intergenic	8	Various phenotypes	D10S1786	10q23.1	1.65	7.68[b]†
rs4933526	10q23.31	1.02×10^{-05}	RP11-478K7.1	intergenic	5	LCA migraine	D10S2327	10q22.3	11.39	2.32[b]
rs4933526	10q23.31	1.02×10^{-05}	RP11-478K7.1	intergenic	8	Various phenotypes	D10S1786	10q23.1	7.85	7.68[b] †
rs986222	10q23.31	3.37×10^{-06}	AL139340.2	intergenic	5	LCA migraine	D10S2327	10q22.3	11.54	2.32[b]
rs986222	10q23.31	3.37×10^{-06}	AL139340.2	intergenic	8	Various phenotypes	D10S1786	10q23.1	8.00	7.68[b]†
rs1946047	11q24.2	5.00×10^{-05}	PRR10	intergenic	9	MA	D11S4464	11q24.1	3.05	5.6[a]

Table 9.5 (continued)

rs2722223	12q21.33	5.78 x 10^{-05}	AC009522.1	intergenic	1	aggravation	D12S1064	12q21.33	0.59	HLOD 2.17[c]
rs5028961	12q23.3	5.85 x 10^{-05}	TXNRD1	intergenic	1	aggravation	D12S1064	12q21.33	13.97	HLOD 2.17[c]
rs6539150	12q23.3	2.08 x 10^{-05}	CHST11	intergenic	1	aggravation	D12S1064	12q21.33	13.98	HLOD 2.17[c]
rs11851709	14q32.2	4.10 x 10^{-05}	AL162151.1	intergenic	10	FHM/MO/MA	rs1054195	14q32.13	3.73	3.83[c]
rs11247555	17p13.3	7.08 x 10^{-05}	VPS53	intronic	1	Pulsation	D17S945	17p13.1	9.30	HLOD 4.65[c]
rs9913267	17p13.3	2.71 x 10^{-05}	GARNL4	intronic	1	Pulsation	D17S945	17p13.1	6.97	HLOD 4.65[c]
rs6066559	20q13.13	6.08 x 10^{-05}	AL357558.1	intergenic	11	MO	D20S96	20q13.11	4.48	1.6[b]
rs6107848	20p12.3	5.90 x 10^{-06}	AL121911.1	intergenic	6	LCA migraine	D20S112	20p12.1	10.72	1.85[b]
rs6107848	20p12.3	5.90 x 10^{-06}	AL121911.1	intergenic	4	migraine/bipolar	D20S470	20p12.1	10.78	1.95[b]
rs979012	20p12.3	9.74 x 10^{-05}	AL035668.1	intergenic	6	LCA migraine	D20S112	20p12.1	10.69	1.85[b]
rs979012	20p12.3	9.74 x 10^{-05}	AL035668.1	intergenic	4	migraine/bipolar	D20S470	20p12.1	10.75	1.95[b]

Overview of results overlapping with previously reported linkage regions. First, the best SNP per region with p-value < 1 x 10^{-4} was selected. These were compared with linkage results from genomewide studies published between 2002 and 2010. Results were included if the SNP was located within 15Mb from a reported linkage region. Positions are based on NCBI build 36.

*Numbers refer to the following studies: 1 Anttila et al., (2006); 2 Björnsson et al., (2003); 3 Wessman et al., (2002); 4 Oedegaard et al., (2009); 5 Nyholt et al., (2005); 6 Ligthart et al., (2008); 7 Carlsson et al., (2002); 8 Anttila et al., (2008); 9 Cader et al., (2003); 10 Cuenca-León et al., (2009); 11 Björnsson et al., (2003).

[a] Multipoint parametric; [b] Multipoint nonparametric; [c] Two-point parametric; † Highest LOD score detected using multiple phenotypes; MO = migraine without aura, MA = migraine with aura, FHM = familial hemiplegic migraine, HLOD = LOD score under locus heterogeneity.

An important strength of this study also relates to the population-based design. Because the samples are unselected with respect to phenotype, the observed migraine cases are expected to be representative of migraine in the general population. In clinic-based studies, there is a considerable risk of confounding due to high prevalence of comorbid disorders. Another strength is that all participants in this study were of western-European ancestry. Although there may be some heterogeneity between the Icelandic sample and the genetic isolate of the ERF study and the rest of the samples, heterogeneity between the population-based Dutch samples will be limited.

In conclusion, this study provides strong evidence for the involvement of the *NGFR* gene in common migraine, based on the results of six independent samples of European ancestry, which all showed an effect in the same direction. *NGFR* is a strong candidate not only because a SNP in this gene showed the most significant association with migraine, but also based on the existing literature and knowledge on the pathways involved in migraine. Additional prioritization based on results of previous association and linkage studies revealed that *ATP1A2* and *GRID2* may be involved in migraine. Functional studies are needed to confirm that these genes are indeed causally related to migraine, and to elucidate what their role is in the mechanisms underlying migraine.

References

Andreou, A. P., & Goadsby, P. J. (2009). Therapeutic potential of novel glutamate receptor antagonists in migraine. Expert Opin Investig Drugs, 18(6), 789-803.

Anttila, V., Kallela, M., Oswell, G., Kaunisto, M. A., Nyholt, D. R., Hamalainen, E., et al. (2006). Trait components provide tools to dissect the genetic susceptibility of migraine. Am J Hum Genet, 79(1), 85-99.

Anttila, V., Nyholt, D. R., Kallela, M., Artto, V., Vepsalainen, S., Jakkula, E., et al. (2008). Consistently replicating locus linked to migraine on 10q22-q23. Am J Hum Genet, 82(5), 1051-1063.

Asuni, C., Stochino, M. E., Cherchi, A., Manchia, M., Congiu, D., Manconi, F., et al. (2009). Migraine and tumour necrosis factor gene polymorphism. An association study in a Sardinian sample. J Neurol, 256(2), 194-197.

Aulchenko, Y. S., Ripke, S., Isaacs, A., & van Duijn, C. M. (2007). GenABEL: an R library for genome-wide association analysis. Bioinformatics, 23(10), 1294-1296.

Bjornsson, A., Gudmundsson, G., Gudfinnsson, E., Hrafnsdottir, M., Benedikz, J., Skuladottir, S., et al. (2003). Localization of a gene for migraine without aura to chromosome 4q21. Am J Hum Genet, 73(5), 986-993.

Blandini, F., Rinaldi, L., Tassorelli, C., Sances, G., Motta, M., Samuele, A., et al. (2006). Peripheral levels of BDNF and NGF in primary headaches. Cephalalgia, 26(2), 136-142.

Boomsma, D. I., de Geus, E. J., Vink, J. M., Stubbe, J. H., Distel, M. A., Hottenga, J. J., et al. (2006). Netherlands Twin Register: from twins to twin families. Twin Res Hum Genet, 9(6), 849-857.

Boomsma, D. I., Willemsen, G., Sullivan, P. F., Heutink, P., Meijer, P., Sondervan, D., et al. (2008). Genome-wide association of major depression: description of samples for the GAIN Major Depressive Disorder Study: NTR and NESDA biobank projects. Eur J Hum Genet, 16(3), 335-342.

Cader, Z. M., Noble-Topham, S., Dyment, D. A., Cherny, S. S., Brown, J. D., Rice, G. P., et al. (2003). Significant linkage to migraine with aura on chromosome 11q24. Hum Mol Genet, 12(19), 2511-2517.

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

Colson, N. J., Fernandez, F., Lea, R. A., & Griffiths, L. R. (2007). The search for migraine genes: an overview of current knowledge. Cell Mol Life Sci, 64(3), 331-344.

Colson, N. J., Lea, R. A., Quinlan, S., MacMillan, J., & Griffiths, L. R. (2004). The estrogen receptor 1 G594A polymorphism is associated with migraine susceptibility in two independent case/control groups. Neurogenetics, 5(2), 129-133.

Colson, N. J., Lea, R. A., Quinlan, S., MacMillan, J., & Griffiths, L. R. (2005). Investigation of hormone receptor genes in migraine. Neurogenetics, 6(1), 17-23.

Corominas, R., Ribases, M., Cuenca-Leon, E., Narberhaus, B., Serra, S. A., del Toro, M., et al. (2009). Contribution of syntaxin 1A to the genetic susceptibility to migraine: a case-control association study in the Spanish population. Neurosci Lett, 455(2), 105-109.

Corominas, R., Sobrido, M. J., Ribases, M., Cuenca-Leon, E., Blanco-Arias, P., Narberhaus, B., et al. (2010). Association study of the serotoninergic system in migraine in the spanish population. Am J Med Genet B Neuropsychiatr Genet, 153B(1), 177-184.

Cuenca-Leon, E., Corominas, R., Montfort, M., Artigas, J., Roig, M., Bayes, M., et al. (2009). Familial hemiplegic migraine: linkage to chromosome 14q32 in a Spanish kindred. Neurogenetics, 10(3), 191-198.

De Fusco, M., Marconi, R., Silvestri, L., Atorino, L., Rampoldi, L., Morgante, L., et al. (2003). Haploinsufficiency of ATP1A2 encoding the Na+/K+ pump alpha2 subunit associated with familial hemiplegic migraine type 2. Nat Genet, 33(2), 192-196.

De Vries, B., Frants, R. R., Ferrari, M. D., & van den Maagdenberg, A. M. (2009). Molecular genetics of migraine. Hum Genet, 126(1), 115-132.

Dichgans, M., Freilinger, T., Eckstein, G., Babini, E., Lorenz-Depiereux, B., Biskup, S., et al. (2005). Mutation in the neuronal voltage-gated sodium channel SCN1A in familial hemiplegic migraine. Lancet, 366(9483), 371-377.

Distel, M. A., Ligthart, L., Willemsen, G., Nyholt, D. R., Trull, T. J., & Boomsma, D. I. (2007). Personality, Health and Lifestyle in a Questionnaire Family Study: A Comparison Between Highly Cooperative and Less Cooperative Families. Twin Res Hum Genet, 10(2), 348-353.

Dudbridge, F., & Gusnanto, A. (2008). Estimation of significance thresholds for genomewide association scans. Genet Epidemiol, 32(3), 227-234.

Erdal, M. E., Herken, H., Yilmaz, M., & Bayazit, Y. A. (2001). Association of the T102C polymorphism of 5-HT2A receptor gene with aura in migraine. J Neurol Sci, 188(1-2), 99-101.

Fernandez, F., Colson, N., Quinlan, S., MacMillan, J., Lea, R. A., & Griffiths, L. R. (2009). Association between migraine and a functional polymorphism at the dopamine beta-hydroxylase locus. Neurogenetics, 10(3), 199-208.

Fukui, Y., Ohtori, S., Yamashita, M., Yamauchi, K., Inoue, G., Suzuki, M., et al. (2010). Low affinity NGF receptor (p75 neurotrophin receptor) inhibitory antibody reduces pain behavior and CGRP expression in DRG in the mouse sciatic nerve crush model. J Orthop Res, 28(3), 279-283.

Harris, T. B., Launer, L. J., Eiriksdottir, G., Kjartansson, O., Jonsson, P. V., Sigurdsson, G., et al. (2007). Age, Gene/Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. Am J Epidemiol, 165(9), 1076-1087.

Headache Classification Committee of the International Headache Society (2004). The International Classification of Headache Disorders: 2nd edition, Cephalalgia (Vol. 24 [Suppl 1], pp. 9-160).

Hofman, A., Breteler, M. M., van Duijn, C. M., Krestin, G. P., Pols, H. A., Stricker, B. H., et al. (2007). The Rotterdam Study: objectives and design update. Eur J Epidemiol, 22(11), 819-829.

Jonsdottir, L. S., Sigfusson, N., Gudnason, V., Sigvaldason, H., & Thorgeirsson, G. (2002). Do lipids, blood pressure, diabetes, and smoking confer equal risk of myocardial infarction in women as in men? The Reykjavik Study. J Cardiovasc Risk, 9(2), 67-76.

Kara, I., Sazci, A., Ergul, E., Kaya, G., & Kilic, G. (2003). Association of the C677T and A1298C polymorphisms in the 5,10 methylenetetrahydrofolate reductase gene in patients with migraine risk. Brain Res Mol Brain Res, 111(1-2), 84-90.

Kowa, H., Fusayasu, E., Ijiri, T., Ishizaki, K., Yasui, K., Nakaso, K., et al. (2005). Association of the insertion/deletion polymorphism of the angiotensin I-converting enzyme gene in patients of migraine with aura. Neurosci Lett, 374(2), 129-131.

Kowa, H., Yasui, K., Takeshima, T., Urakami, K., Sakai, F., & Nakashima, K. (2000). The homozygous C677T mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for migraine. Am J Med Genet, 96(6), 762-764.

Kusumi, M., Ishizaki, K., Kowa, H., Adachi, Y., Takeshima, T., Sakai, F., et al. (2003). Glutathione S-transferase polymorphisms: susceptibility to migraine without aura. Eur Neurol, 49(4), 218-222.

Launer, L. J., Terwindt, G. M., & Ferrari, M. D. (1999). The prevalence and characteristics of migraine in a population-based cohort: the GEM study. Neurology, 53(3), 537-542.

Lea, R. A., Dohy, A., Jordan, K., Quinlan, S., Brimage, P. J., & Griffiths, L. R. (2000). Evidence for allelic association of the dopamine beta-hydroxylase gene (DBH) with susceptibility to typical migraine. Neurogenetics, 3(1), 35-40.

Lea, R. A., Ovcaric, M., Sundholm, J., MacMillan, J., & Griffiths, L. R. (2004). The methylenetetrahydrofolate reductase gene variant C677T influences susceptibility to migraine with aura. BMC Med, 2, 3.

Lee, K. A., Jang, S. Y., Sohn, K. M., Won, H. H., Kim, M. J., Kim, J. W., et al. (2007). Association between a polymorphism in the lymphotoxin-a promoter region and migraine. Headache, 47(7), 1056-1062.

Ligthart, L., Nyholt, D. R., Hottenga, J. J., Distel, M. A., Willemsen, G., & Boomsma, D. I. (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, 147B(7), 1186-1195.

Marchini, J., Howie, B., Myers, S., McVean, G., & Donnelly, P. (2007). A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet, 39(7), 906-913.

Marziniak, M., Mossner, R., Schmitt, A., Lesch, K. P., & Sommer, C. (2005). A functional serotonin transporter gene polymorphism is associated with migraine with aura. Neurology, 64(1), 157-159.

McCarthy, L. C., Hosford, D. A., Riley, J. H., Bird, M. I., White, N. J., Hewett, D. R., et al. (2001). Single-nucleotide polymorphism alleles in the insulin receptor gene are associated with typical migraine. Genomics, 78(3), 135-149.

Mochi, M., Cevoli, S., Cortelli, P., Pierangeli, G., Scapoli, C., Soriani, S., et al. (2003). Investigation of an LDLR gene polymorphism (19p13.2) in susceptibility to migraine without aura. J Neurol Sci, 213(1-2), 7-10.

Nyholt, D. R., Morley, K. I., Ferreira, M. A., Medland, S. E., Boomsma, D. I., Heath, A. C., et al. (2005). Genomewide significant linkage to migrainous headache on chromosome 5q21. Am J Hum Genet, 77(3), 500-512.

Oedegaard, K. J., Greenwood, T. A., Lunde, A., Fasmer, O. B., Akiskal, H. S., & Kelsoe, J. R. (2009). A genome-wide linkage study of bipolar disorder and co-morbid migraine: Replication of migraine linkage on chromosome 4q24, and suggestion of an overlapping susceptibility region for both disorders on chromosome 20p11. J Affect Disord.

Ophoff, R. A., Terwindt, G. M., Vergouwe, M. N., van Eijk, R., Oefner, P. J., Hoffman, S. M., et al. (1996). Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell, 87(3), 543-552.

Oterino, A., Pascual, J., Ruiz de Alegria, C., Valle, N., Castillo, J., Bravo, Y., et al. (2006). Association of migraine and ESR1 G325C polymorphism. Neuroreport, 17(1), 61-64.

Paterna, S., Di Pasquale, P., Cottone, C., Seidita, G., Cardinale, A., Parrinello, G., et al. (1997). Migraine without aura and ACE-gene deletion polymorphism: is there a correlation? Preliminary findings. Cardiovasc Drugs Ther, 11(4), 603-604.

Paterna, S., Di Pasquale, P., D'Angelo, A., Seidita, G., Tuttolomondo, A., Cardinale, A., et al. (2000). Angiotensin-converting enzyme gene deletion polymorphism determines an increase in frequency of migraine attacks in patients suffering from migraine without aura. Eur Neurol, 43(3), 133-136.

Penninx, B. W., Beekman, A. T., Smit, J. H., Zitman, F. G., Nolen, W. A., Spinhoven, P., et al. (2008). The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res, 17(3), 121-140.

Peroutka, S. J., Wilhoit, T., & Jones, K. (1997). Clinical susceptibility to migraine with aura is modified by dopamine D2 receptor (DRD2) NcoI alleles. Neurology, 49(1), 201-206.

Pezet, S., & McMahon, S. B. (2006). Neurotrophins: mediators and modulators of pain. Annu Rev Neurosci, 29, 507-538.

Rainero, I., Grimaldi, L. M., Salani, G., Valfre, W., Rivoiro, C., Savi, L., et al. (2004). Association between the tumor necrosis factor-alpha -308 G/A gene polymorphism and migraine. Neurology, 62(1), 141-143.

Rubino, E., Ferrero, M., Rainero, I., Binello, E., Vaula, G., & Pinessi, L. (2009). Association of the C677T polymorphism in the MTHFR gene with migraine: a meta-analysis. Cephalalgia, 29(8), 818-825.

Santos, R. L., Zillikens, M. C., Rivadeneira, F. R., Pols, H. A., Oostra, B. A., van Duijn, C. M., et al. (2006). Heritability of fasting glucose levels in a young genetically isolated population. Diabetologia, 49(4), 667-672.

Sarchielli, P., Alberti, A., Floridi, A., & Gallai, V. (2001). Levels of nerve growth factor in cerebrospinal fluid of chronic daily headache patients. Neurology, 57(1), 132-134.

Scher, A. I., Terwindt, G. M., Verschuren, W. M., Kruit, M. C., Blom, H. J., Kowa, H., et al. (2006). Migraine and MTHFR C677T genotype in a population-based sample. Ann Neurol, 59(2), 372-375.

Scher, A. I., Gudmundsson, L. S., Sigurdsson, S., Ghambaryan, A., Aspelund, T., Eiriksdottir, G., et al. (2009). Migraine headache in middle age and late-life brain infarcts. Jama, 301(24), 2563-2570.

Schurks, M., Zee, R. Y., Buring, J. E., & Kurth, T. (2008). Interrelationships among the MTHFR 677C>T polymorphism, migraine, and cardiovascular disease. Neurology, 71(7), 505-513.

Schwaag, S., Evers, S., Schirmacher, A., Stogbauer, F., Ringelstein, E. B., & Kuhlenbaumer, G. (2006). Genetic variants of the NOTCH3 gene in migraine--a mutation analysis and association study. Cephalalgia, 26(2), 158-161.

Sigurdsson, E., Thorgeirsson, G., Sigvaldason, H., & Sigfusson, N. (1995). Unrecognized myocardial infarction: epidemiology, clinical characteristics, and the prognostic role of angina pectoris. The Reykjavik Study. Ann Intern Med, 122(2), 96-102.

Sleegers, K., de Koning, I., Aulchenko, Y. S., van Rijn, M. J., Houben, M. P., Croes, E. A., et al. (2007). Cerebrovascular risk factors do not contribute to genetic variance of cognitive function: the ERF study. Neurobiol Aging, 28(5), 735-741.

Stam, A. H., de Vries, B., Janssens, A. C., Vanmolkot, K. R., Aulchenko, Y. S., Henneman, P., et al. (2010). Shared genetic factors in migraine and depression. Evidence from a genetic isolate. Neurology, 74(4), 288-294.

Stewart, W. F., Lipton, R. B., Celentano, D. D., & Reed, M. L. (1992). Prevalence of migraine headache in the United States. Relation to age, income, race, and other sociodemographic factors. Jama, 267(1), 64-69.

Tikka-Kleemola, P., Kaunisto, M. A., Hamalainen, E., Todt, U., Gobel, H., Kaprio, J., et al. (2009). Genetic association study of endothelin-1 and its receptors EDNRA and EDNRB in migraine with aura. Cephalalgia, 29(11), 1224-1231.

Todt, U., Dichgans, M., Jurkat-Rott, K., Heinze, A., Zifarelli, G., Koenderink, J. B., et al. (2005). Rare missense variants in ATP1A2 in families with clustering of common forms of migraine. Hum Mutat, 26(4), 315-321.

Todt, U., Netzer, C., Toliat, M., Heinze, A., Goebel, I., Nurnberg, P., et al. (2009). New genetic evidence for involvement of the dopamine system in migraine with aura. Hum Genet, 125(3), 265-279.

Trabace, S., Brioli, G., Lulli, P., Morellini, M., Giacovazzo, M., Cicciarelli, G., et al. (2002). Tumor necrosis factor gene polymorphism in migraine. Headache, 42(5), 341-345.

Tzourio, C., El Amrani, M., Poirier, O., Nicaud, V., Bousser, M. G., & Alperovitch, A. (2001). Association between migraine and endothelin type A receptor (ETA -231 A/G) gene polymorphism. Neurology, 56(10), 1273-1277.

Van den Maagdenberg, A. M., Haan, J., Terwindt, G. M., & Ferrari, M. D. (2007). Migraine: gene mutations and functional consequences. Curr Opin Neurol, 20(3), 299-305.

Visscher, P. M. (2008). Sizing up human height variation. Nat Genet, 40(5), 489-490.

Wessman, M., Kallela, M., Kaunisto, M. A., Marttila, P., Sobel, E., Hartiala, J., et al. (2002). A susceptibility locus for migraine with aura, on chromosome 4q24. Am J Hum Genet, 70(3), 652-662.

Yilmaz, M., Erdal, M. E., Herken, H., Cataloluk, O., Barlas, O., & Bayazit, Y. A. (2001). Significance of serotonin transporter gene polymorphism in migraine. J Neurol Sci, 186(1-2), 27-30.

Zhang, Y. H., & Nicol, G. D. (2004). NGF-mediated sensitization of the excitability of rat sensory neurons is prevented by a blocking antibody to the p75 neurotrophin receptor. Neurosci Lett, 366(2), 187-192.

10

Summary & General Discussion

The research described in this thesis focuses on the definition and etiology of migraine in large population based studies. A variety of methods were applied in this work. To optimize the definition of the migraine phenotype, we applied latent class analysis to data collected in survey studies. Genetic modelling of twin data was applied to examine the comorbidity of migraine with depression and the heritability of migraine as a function of depression status. Finally, linkage and genome-wide association studies were performed to localize and identify genetic variants potentially involved in migraine. In this chapter, I first provide a summary of the main results followed by a discussion of the implications of this work. I conclude with an outline of recommendations for future research.

SUMMARY

INTRODUCTORY CHAPTERS

In the first chapter of this thesis, an overview is provided of the literature on the epidemiology and pathophysiology of migraine. The chapter describes how migraine is currently diagnosed, what is known about the mechanisms underlying a migraine attack, and how migraine is associated with several psychiatric and non-psychiatric conditions. In addition, the chapter provides a summary of the most important results from migraine gene-finding studies, which are primarily based on studies of *familial hemiplegic migraine*, a rare and severe monogenic subtype of migraine with aura. Three genes have been implicated in this disorder, all of which are involved in ion transport. For common migraine, several linkage and candidate-gene association studies have been performed, but a causal genetic variant has not been identified. The chapter ends with an outline of the research described in this thesis. Important aims were to investigate the genetic architecture of migraine and comorbid depression, and to identify the genes underlying migraine.

Chapter 2 provides an overview of the methods employed in genetic epidemiology to address these issues. These methods include the genetic modelling of twin and family data, genetic linkage studies in family data, genome-wide association studies in (unrelated) cases and controls and meta-analysis of the results of such studies,

Chapters 3 and 4 describe the data collection procedures in the Netherlands Twin Register (NTR) that preceded the studies described in this thesis, and provide some background information on the samples analyzed. In Chapter 4, it was investigated whether there was any evidence for non-random participation in the surveys of the NTR. This study concluded that there was no evidence of a response bias with respect to migraine status.

ANALYSES OF THE MIGRAINE PHENOTYPE AND COMORBIDITY WITH DEPRESSION

Chapters 5-7 focused on the analysis of the migraine phenotype by analyzing the symptoms of migraine, as defined by the official diagnostic criteria (IHS; Headache Classification Committee of the International Headache Society, 2004), the comorbidity of migraine and depression and the moderation of the heritability of migraine by depression. Data on migraine and depression were collected with mailed surveys in large samples of NTR participants. Additional

data on major depressive disorder from the Netherlands Study of Depression and Anxiety (NESDA; Penninx et al., 2008) were included in Chapter 6.

In Chapter 5, migraine symptoms were analyzed with latent class analysis (LCA) to investigate whether there was any evidence for distinct subtypes of migraine. One important question was whether distinct classes would be characterized by the presence or absence of aura symptoms. The LCA empirically identified 4 classes of individuals, based on migraine symptomatology. The results were very similar for males and females, indicating that although the prevalence is higher in females, there are no qualitative sex differences in migraine symptomatology. The frequency of aura symptoms was not a particularly distinctive feature that characterized any of the groups. The observed pattern showed that what distinguished the classes was the severity of the migraine and the number of symptoms reported, rather than qualitative differences. The only qualitative difference between the classes was that the increase in symptom prevalences in class 3 compared to class 2 was relatively stronger for the symptoms *photo-/phonophobia*, *nausea/vomiting*, and *aura*, reflecting the fact that these symptoms are relatively rare in mildly affected patients. But even in the most severely affected group (class 3) only 49% of the patients had aura. In this study, the heritability of the LCA-based phenotype was estimated at 50%, which is comparable to the heritability of migraine according to IHS-criteria (49%). Due to the low number of dizygotic male twin pairs screening positive in the sixth survey, the heritability estimates reported in Chapter 5 were based on data from women only. In Chapter 7, the heritability of LCA-derived migraine was estimated at 45%, based on data from both women and men.

In summary, the results indicate that no distinct group of MA patients can be identified based on symptomatology. This is consistent with the results of an earlier study in Australian twins (Nyholt et al., 2004), and suggests a similar disease process may underlie MO and MA. The results of this chapter support the use of a broad, LCA-based phenotype in genetic studies of migraine, and the combined analysis of data from patients with MO and MA. This results in a larger number of individuals classified as affected, and thus maximizes power to detect genetic effects. In the gene-finding studies described in Chapter 8 and 9, this strategy was continued.

In Chapter 6 migraine symptomatology in MDD patients and non-depressed controls was compared using a similar LCA-based approach. It is known that migraine has a higher prevalence in depressed patients (Breslau et al., 2000; Merikangas et al., 1988). However, the diagnostic criteria allow a

substantial heterogeneity of specific symptoms between individuals (i.e., many different combinations of symptoms can result in the same endpoint diagnosis). Therefore it is important to know whether we are indeed observing the same disorder in depressed and non-depressed individuals. Because certain symptoms are more prevalent in patients with severe migraine (Chapter 5), a higher prevalence of severe migraine in MDD patients might be mistaken for a qualitative difference in migraine symptomatology, for instance because MDD patients more often have photo-/phonophobia or aura. For this reason, we examined whether there were qualitative differences, while taking differences in prevalence and severity into account. This was achieved by performing a multi-group latent class analysis, in which symptom profiles were estimated in MDD-patients and non-depressed controls. It was formally tested whether the same symptom profiles were observed in the MDD patients and the controls.

The data confirmed that the prevalence of severe migraine was much higher in the MDD patients. However, qualitative differences between the symptom profiles of MDD patients and controls were only minor: the symptoms aggravation by physical activity and aura both had a slightly higher prevalence in the MDD group, for the other symptoms no significant differences were found between the two groups. These results suggest that a similar disease process may underlie migraine in individuals with and without MDD. However, a similar symptomatology does not prove that the etiology of the disorder is the same. Thus, while there is migraine is qualitatively similar in depressed and non-depressed individuals, we should be cautious in assuming the same etiology in both groups.

In Chapter 7 we took the investigation of migraine and comorbid depression one step further and examined 1) the genetic and environmental correlation between migraine and anxious depression, 2) the genetic architecture of migraine in individuals with high and low anxious depression scores, and 3) tested whether the association is more likely explained by causality or by shared underlying genes (pleiotropy). Firstly, the heritability estimates for migraine and depression were 45% and 55%, respectively, and a bivariate genetic model showed that the two traits were indeed genetically correlated. The non-shared environmental factors (which explained the remaining variance in both traits) were also correlated. Secondly, a test of the moderating effects of depression on the heritability of migraine, revealed that migraine was more heritable in individuals with low anxious depression scores. Thirdly, in MZ twin pairs discordant for migraine, the twin without migraine did not have an increased risk of depression, and vice versa. In other words, the fact

that the first twin had migraine, did not increase the risk that the second twin had depression, unless the second twin also had migraine (and vice versa). If there was no causality, it would be expected that the MZ co-twin without migraine had the same risk of depression as the twin with migraine, because they share the same genes. These results suggest that the association between migraine and anxious depression is most likely explained by bidirectional causality.

Gene-finding studies

Given the heritability of migraine, a logical next step is to try and identify the genes that underlie the disorder. This work is summarized in Chapters 8 and 9, which describe linkage and genome-wide association studies for migraine. Following from the results of Chapter 5, linkage and genome-wide association analyses these studies were performed using the LCA-based classification of migraine, in order to maximize the power to detect migraine susceptibility genes.

In the linkage study described in Chapter 8 three linkage peaks were identified that surpassed the threshold for suggestive linkage, on chromosome 1q23, 13q32 and 20p12. The finding on chromosome 1 was particularly interesting because it was located only 5 cM away from the ATPase, Na+/K+ transporting, alpha 2 polypeptide *(ATP1A2)* gene, which is involved in familial hemiplegic migraine. In addition, some smaller linkage peaks were identified, which replicated previous findings on chromosome 5q21 (Nyholt et al., 2005) and 10q22 (Anttila et al., 2006; Nyholt et al., 2005). Interestingly, the peak on chromosome 20 was recently replicated in a linkage study of bipolar disorder and comorbid migraine in families from the NIMH Bipolar Genetics Initiative, where it was linked to both migraine and bipolar disorder (Oedegaard et al., 2009).

Chapter 9 describes the results of a meta-analysis of genome-wide association studies for migraine in six samples of European ancestry (five Dutch samples and one Icelandic). The best result in this study was found for a SNP on chromosome 17, located in the *NGFR* gene. This gene codes for the neural growth factor (NGF) receptor, which plays an important role in pain transmission and sensitization. NGF is upregulated in many chronic pain conditions (Pezet & McMahon, 2006) and has been associated with headache in studies that showed altered cerebrospinal fluid and platelet levels of NGF in headache patients (Blandini et al., 2006; Sarchielli et al., 2001). However, the NGF receptor has not been implicated in migraine etiology before. The results

of the meta-analysis were also compared to previous linkage and association studies, which revealed the possible involvement of *ATP1A2* (one of the genes that causes FHM) and the glutamate receptor, ionotropic, delta 2 *(GRID2)* gene in common migraine. The latter is an interesting candidate because it is located in a region on chromosome 4q22 that has been reported in several independent linkage studies (Anttila et al., 2006; Bjornsson et al., 2003; Oedegaard et al., 2009; Wessman et al., 2002). Glutamate is thought to play an important role in migraine, possibly because high extracellular levels of glutamate can facilitate cortical spreading depression, the mechanism that underlies migraine aura (Sanchez-Del-Rio et al., 2006; van den Maagdenberg et al., 2007).

DISCUSSION

MIGRAINE ASSESSMENT

The assessment of migraine in most studies in this thesis is based on survey data. In large population based studies, assessment of each individual participant by a neurologist (generally perceived to be the 'gold standard' for migraine diagnosis) is not feasible due to financial and time constraints. Genetic studies require large samples and survey-based assessment is a good alternative method that allows the phenotyping of large numbers of participants, so that large-scale genetic epidemiological studies become feasible.

The survey used to assess migraine (Table 3.2) has been described extensively in Chapter 3. It includes items on eight of the symptoms that are included in the official diagnostic criteria (Headache Classification Committee of the International Headache Society, 2004). As described in Chapter 3, the test-retest reliability of the headache items was good, with high correlations between survey 6 and 7 (.82 - .87 for the different items), and also between the first wave of survey 7 (November 2004) and a shortened version of the survey sent to a selected group of participants in July 2005 (correlations .82 - .92). A limitation of the questionnaire was that no question on unilaterality of the headache was included. In addition, photo-, phono-, and osmophobia were combined into one question. To approximate an IHS diagnosis, we considered the C criterion (see Table 1.1) valid only if two out of the three measured C-items (moderate/severe pain intensity, pulsating quality and aggravation by physical activity) were present, which is more stringent than the official requirement (two out of four). The photo- and phonophobia requirement was less stringent

than the official criteria specify. The overall prevalence estimates of IHS migraine in our sample (4% in males, 13% in females) are slightly lower than what is found in other studies based on IHS criteria (e.g., Stewart et al., 1992), suggesting that, due to our relatively strict definition, the prevalence of IHS migraine was underestimated. The number of false positive diagnoses based on this definition is most likely limited.

Classification of participants with Latent Class Analysis

Latent class analysis was applied to investigate whether subgroups of migraine patients could be identified, and whether separate MO and MA subtypes existed. The result was a classification in which the different subtypes differed primarily in terms of severity of the migraine headaches. Class 0 is the group of unaffected individuals, class 1 individuals have mild, non-migrainous headaches, class 2 can be described as moderate migrainous headache, and the individuals in class 3 have severe migrainous headache.

In the gene-finding studies that followed (Chapters 8 and 9), both class 2 and class 3 individuals were classified as migraineurs, which resulted in a high prevalence of migrainous headache (13% in males, 35% in females). As I have argued in previous chapters, using a broad phenotype definition has the advantage that more potentially genetically informative individuals are classified as affected, which increases the power to detect genetic effects. But does a broader definition still reflect migraine, or does it result in the inclusion of other types of mild headache as well?

First, as shown in Chapter 8, the heritability of migraine based on LCA ('LCA migraine'; $h^2 = 49\%$) is approximately the same as the heritability of migraine according to ICHD-II criteria ('IHS migraine'; $h^2 = 46\%$). This indicates that by using the broader phenotype, we do not lose any genetic information. In an Australian study that applied the same method, the heritability of LCA and IHS migraine were also similar, although slightly lower than in our study (40% and 36%, respectively). These estimates are similar to what is generally found for migraine. For example, a study of almost 30,000 twins from six European countries estimated the heritability of migraine at 46% (Mulder et al., 2003).

Second, in the linkage study (Chapter 8), we observed that when we used IHS migraine or 'LCA-severe' (i.e. class 3) as the phenotype, linkage peaks detected with the phenotype based on LCA class 2 + 3 generally became smaller. The use of a stricter definition did not identify any peaks that had not been detected using the more liberal LCA definition (see Figure 8.2). The GWA

analyses for Chapter 9 were also conducted with both IHS and LCA migraine as the phenotypes. The associations detected with the LCA phenotype were still present when using the IHS migraine phenotype, but in general, the p-values were markedly less significant. No strong association signals were observed that emerged only when using the more stringent IHS phenotype.

Clearly, the fact that using a more stringent phenotype reduces the number of cases largely explains the finding that using LCA migraine as the phenotype produces better results. However, if the LCA migraine phenotype were a mixture of migraine and a different type of 'general, undefined headache', one would expect an increase in genetic heterogeneity that would be unlikely to result in more significant association signals than a strict IHS migraine phenotype. This strengthens my confidence that by using the broad LCA phenotype, we indeed measure a genetic risk of migraine. In addition, it is always possible to check the validity of findings based on LCA migraine by repeating the analysis in the subset of individuals fulfilling strict IHS criteria for migraine. In summary, our results indicate that LCA-based classification is a valuable method for large-scale population based genetic studies of migraine, where power and sample size are generally the limiting factors.

Screening procedure

To lower the burden for participants, it is common to start a questionnaire with a screening question, and ask the participant to complete the remaining questions only if these are relevant given the outcome of the screening question. In our migraine questionnaire, the screening question was "Do you ever experience headache attacks, for instance migraine?". Approximately 30% of participants screen positive, based on this question. Thus, with no other information present, we have to assume that the remaining 70% never have headache attacks. This is a high percentage compared to, for instance, a similar study by Nyholt et al. (2004; see also Chapter 5). However, it is possible that due to the phrasing of the question, individuals with mild headaches will think their condition (which does not compare to a severe condition like migraine) does not qualify to even be mentioned. Also, headaches that do not occur attack-wise might go unnoticed with this type of screening. This may explain why latent class 1 had a relatively low prevalence under the 4-class LCA model (Chapter 5); many individuals with this mild type of headache may have screened negative. On the other hand, this also indicates that individuals with moderate or severe migrainous headache (which is our primary interest) are unlikely to screen negative.

A consequence of the use of screening questions is that the collected data are essentially censored, and that there may be some individuals in the 'unaffected' group who have a minor genetic risk of headache. These individuals are not ideal controls, because they are not entirely unaffected.

In the linkage study (Chapter 8), the screening procedure was not an issue, because in this study an affected sib-pair design was used. Thus, the results were based only on individuals we were quite confident were affected, and not on controls who might potentially carry a minor genetic risk of migraine. In the GWA study described in Chapters 9, the unaffected individuals were included as controls. In these studies, we treated the class 1 individuals as unaffected, based on the fact that on average, the majority of migraine symptoms were not reported by this group. To assess the potential implications of treating individuals with mild, non-migrainous headaches as controls, we performed an additional analysis in the GAIN sample, in which the phenotypes of the class 1 individuals were set to missing rather than unaffected. This analysis produced very similar results, suggesting the effect of the potential presence of migraine risk genes in these individuals is limited, if present.

The relationship between migraine and depression

In Chapters 5 and 6, no distinct subtypes of migraine were identified, relating to the presence of aura or major depression. The subtypes identified with latent class analysis do not show major qualitative differences but differ primarily in terms of severity. Does this mean there is only one type of migraine? At the phenotypic level, this appears to be the case. However, at the genetic level, there may be differences between groups of patients. Evidence for this hypothesis comes from Chapter 7. In this chapter, migraine was found to be more heritable in non-depressed than in depressed individuals. In addition, risk patterns in relatives of patients with migraine and anxious depression suggested bidirectional causality. If migraine can be causally related to anxious depression in some patients, this could indicate it is genetically different from migraine unrelated to depression.

Interestingly, Merikangas et al. (1993) reported a similar finding concerning risk patterns in relatives of migraine patients. In this study, after controlling for comorbidity, the relatives of probands with migraine had no increased risk of depression, and vice versa. These results were consistent with causality, rather than shared underlying genes (pleiotropy), and the authors suggested that migraine and depression might be syndromically related.

Since this publication, many studies have confirmed that migraine and depression are correlated (e.g., Beghi et al., 2007; Breslau et al., 2000; Mitsikostas & Thomas, 1999; Zwart et al., 2003), and several authors hypothesized that shared genes might be involved (Breslau et al., 1991; Cahill & Murphy, 2004; Frediani & Villani, 2007). However, only few studies actually addressed the underlying mechanisms that might explain the association.

Breslau et al. (2000) published a study which reported a bidirectional relationship between migraine and depression. Migraine predicted first-onset major depression and major depression predicted first-onset migraine. This relationship was specific to migraine: depression did not predict the first onset of other severe headaches.

In 2009, the first twin study was published that explored the hypothesis that the same set of genes may influence both migraine and depression (Schur et al., 2009). In this study, a genetic correlation was indeed reported, a finding we replicated in Chapter 7 of this thesis. Recently, a second study reporting shared genetic factors for migraine and depression was published (Stam et al., 2010). It is worth noting here that the presence of a genetic correlation does not tell us whether the association between two traits is caused by pleiotropy or by a causal mechanism. If the association between A and B is causal, genes that affect A will indirectly also affect B. To decide which hypothesis is more likely, additional information is necessary.

Interestingly, the Schur et al. study included a set of diagrams that showed the risk patterns in co-twins of individuals with migraine, depression, both, or neither. Although these diagrams were not created with the intention to investigate causality, they provide further evidence for a bidirectional causal relationship between migraine and depression. If the first twin had migraine only, the second twin did not have an increased risk of depression, unless they also had migraine, and vice versa. This risk pattern is similar to the pattern observed in Chapter 7 of this thesis, and that observed by Merikangas et al. (1993).

Together, these studies provide considerable evidence supporting the hypothesis of Merikangas et al., that migraine and depression are syndromically related. In other words, migraine might be viewed as part of a 'depression syndrome' in at least a subgroup of patients.

The hypothesis that in some patients, migraine and depression may be syndromically related, raises many new and important questions. For instance, is migraine really an aspect of depression, or is reporting migraine symptoms an aspect of depression? Is the relationship with depression specific to migraine, or

is there an equally strong relationship between depression and other somatic symptoms? Could it be that MDD patients simply overreport somatic symptoms as a result of their depressed mood?

While it is possible that the high prevalence of pain in depressed patients (in part) reflects a report bias, comorbidity of depression and chronic pain has indeed been reported in the literature (Bair et al., 2003). It has been suggested that chronic pain might in fact be a symptom of depression (Lépine & Briley, 2004). Moreover, there is a vast amount of literature that describes the effectiveness of various types of antidepressants in the treatment of headaches (see Tomkins et al. (2001) for a meta-analysis).

Genes for common migraine

What causes common migraine? The linkage study described in this thesis identified new potential regions of interest on chromosome 13 and 20, and replicated previous findings (e.g. the linkage peaks on chromosome 1 and 5), thus strengthening the confidence in these findings. One of our linkage peaks (on chromosome 20) was recently replicated by another group (Oedegaard et al., 2009).

This thesis presents the first meta-analysis of population-based genome-wide association studies on migraine. These analyses, which were based on data from six European cohorts, revealed some promising and plausible candidate genes for common migraine. The best result was found in the *NGFR* gene, which codes for the neural growth factor (NGF) receptor. Strong associations were also found in the *ATP1A2* gene, known to be involved in FHM. The combined results of the GWA meta-analysis and previous linkage studies supported the involvement of *GRID2*, which codes for a glutamate receptor. *GRID2* is located under a linkage peak on chromosome 4q22 that has been reported in several previous studies (Anttila et al., 2006; Bjornsson et al., 2003; Wessman et al., 2002). These promising findings suggest that GWA studies, when sufficiently large, can be quite effective for the identification of migraine genes and especially that combining results from studies using different gene-finding approaches is highly valuable.

Conclusions and implications

The results presented in this thesis, combined with the rapid developments in technology, hold great promise for future research. GWA studies have taught us that complex traits are often affected by large numbers of risk alleles with small effects (Visscher, 2008), which makes large study sizes a necessity. However,

large-scale genotyping will become easier and even more affordable in the future. In addition, the ever-growing sources of information regarding gene function, interactions and biological pathways will provide more tools to help us identify new genes that serve as candidates for migraine. This creates exciting new research opportunities, which will hopefully lead to the identification of actual disease variants, and a more complete understanding of the mechanisms that cause a migraine attack.

But technology is not the only factor that determines the success of gene-finding studies. An equally important aspect of finding genes for complex traits is the choice of a good phenotyping strategy. This is especially important given the potential genetic heterogeneity that may underlie the migraine phenotype. Even familial hemiplegic migraine (FHM), a monogenic disorder which follows a mendelian inheritance pattern, can be caused by dozens of different mutations in at least three different genes (de Vries et al., 2009). Given the complex nature of common migraine, genetic heterogeneity may be even more important in this trait. While the linkage study (Chapter 8) and GWA meta-analysis (Chapter 9) provide evidence for the involvement of one of the FHM genes in common migraine, it is likely that many more genes are involved.

A possible strategy to address the issue of genetic heterogeneity is to carefully document and account for any type of comorbidity in studies of migraine. The diagnostic criteria for migraine (Headache Classification Committee of the International Headache Society, 2004) state that migraine caused by another disorder should be classified as a secondary, rather than a primary headache. Indeed, in neurology, it is not uncommon to think of migraine as a phenomenon that occurs as a consequence of other (neurological) conditions. Examples are cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and retinal vasculopathy with cerebral leukodystrophy (RVCL), both disorders of which migraine is a prominent symptom (de Vries et al., 2009). In psychiatry, on the other hand, migraine is generally viewed as a disorder in itself, which happens to be highly prevalent in psychiatric patients (e.g., Cahill & Murphy, 2004). The possibility that it may actually be secondary to the psychiatric disorder is often neglected, even though it is not a strange thought, given that both neurological and psychiatric disorders are disorders of the brain.

Whether we classify a patient's migraine as a primary or a secondary headache has important implications for the assumptions we make about its etiology. Based on the findings in this thesis, it seems likely that migraine can

be secondary to anxiety and depression, and the same may be true for other psychiatric disorders, or even non-psychiatric comorbidities.

Therefore, it is important to carefully document any type of comorbid pathology in migraine patients. This should enable us to investigate which comorbid conditions are associated with migraine due to pleiotropy, and which conditions might have migraine as one of their symptoms. Furthermore, if all migraine patients were adequately screened for comorbid conditions, this might contribute to a more effective treatment of the migraine, for instance through treatment of the comorbid condition. Eventually, both research and treatment may benefit from such an approach.

REFERENCES

Anttila, V., Kallela, M., Oswell, G., Kaunisto, M. A., Nyholt, D. R., Hamalainen, E., et al. (2006). Trait components provide tools to dissect the genetic susceptibility of migraine. Am J Hum Genet, 79(1), 85-99.

Bair, M. J., Robinson, R. L., Katon, W., & Kroenke, K. (2003). Depression and pain comorbidity: a literature review. Arch Intern Med, 163(20), 2433-2445.

Beghi, E., Allais, G., Cortelli, P., D'Amico, D., De Simone, R., d'Onofrio, F., et al. (2007). Headache and anxiety-depressive disorder comorbidity: the HADAS study. Neurol Sci, 28 Suppl 2, S217-219.

Bjornsson, A., Gudmundsson, G., Gudfinnsson, E., Hrafnsdottir, M., Benedikz, J., Skuladottir, S., et al. (2003). Localization of a gene for migraine without aura to chromosome 4q21. Am J Hum Genet, 73(5), 986-993.

Blandini, F., Rinaldi, L., Tassorelli, C., Sances, G., Motta, M., Samuele, A., et al. (2006). Peripheral levels of BDNF and NGF in primary headaches. Cephalalgia, 26(2), 136-142.

Breslau, N., Davis, G. C., & Andreski, P. (1991). Migraine, psychiatric disorders, and suicide attempts: an epidemiologic study of young adults. Psychiatry Res, 37(1), 11-23.

Breslau, N., Schultz, L. R., Stewart, W. F., Lipton, R. B., Lucia, V. C., & Welch, K. M. (2000). Headache and major depression: is the association specific to migraine? Neurology, 54(2), 308-313.

Cahill, C. M., & Murphy, K. C. (2004). Migraine: another headache for psychiatrists? Br J Psychiatry, 185, 191.

De Vries, B., Frants, R. R., Ferrari, M. D., & van den Maagdenberg, A. M. (2009). Molecular genetics of migraine. Hum Genet, 126(1), 115-132.

Frediani, F., & Villani, V. (2007). Migraine and depression. Neurol Sci, 28 (Suppl 2), S161-165.

Headache Classification Committee of the International Headache Society (2004). The International Classification of Headache Disorders: 2nd edition, Cephalalgia (Vol. 24 [Suppl 1], pp. 9-160).

Lépine, J. P., & Briley, M. (2004). The epidemiology of pain in depression. Hum Psychopharmacol, 19 Suppl 1, S3-7.

Merikangas, K. R., Merikangas, J. R., & Angst, J. (1993). Headache syndromes and psychiatric disorders: association and familial transmission. J Psychiatr Res, 27(2), 197-210.

Merikangas, K. R., Risch, N. J., Merikangas, J. R., Weissman, M. M., & Kidd, K. K. (1988). Migraine and depression: association and familial transmission. J Psychiatr Res, 22(2), 119-129.

Mitsikostas, D. D., & Thomas, A. M. (1999). Comorbidity of headache and depressive disorders. Cephalalgia, 19, 211-217.

Mulder, E. J., Van Baal, C., Gaist, D., Kallela, M., Kaprio, J., Svensson, D. A., et al. (2003). Genetic and environmental influences on migraine: a twin study across six countries. Twin Res, 6(5), 422-431.

Nyholt, D. R., Gillespie, N. G., Heath, A. C., Merikangas, K. R., Duffy, D. L., & Martin, N. G. (2004). Latent class and genetic analysis does not support migraine with aura and migraine without aura as separate entities. Genet Epidemiol, 26(3), 231-244.

Nyholt, D. R., Morley, K. I., Ferreira, M. A., Medland, S. E., Boomsma, D. I., Heath, A. C., et al. (2005). Genomewide significant linkage to migrainous headache on chromosome 5q21. Am J Hum Genet, 77(3), 500-512.

Oedegaard, K. J., Greenwood, T. A., Lunde, A., Fasmer, O. B., Akiskal, H. S., & Kelsoe, J. R. (2009). A genome-wide linkage study of bipolar disorder and co-morbid migraine: Replication of migraine linkage on chromosome 4q24, and suggestion of an overlapping susceptibility region for both disorders on chromosome 20p11. J Affect Disord.

Penninx, B. W., Beekman, A. T., Smit, J. H., Zitman, F. G., Nolen, W. A., Spinhoven, P., et al. (2008). The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res, 17(3), 121-140.

Pezet, S., & McMahon, S. B. (2006). Neurotrophins: mediators and modulators of pain. Annu Rev Neurosci, 29, 507-538.

Sanchez-Del-Rio, M., Reuter, U., & Moskowitz, M. A. (2006). New insights into migraine pathophysiology. Curr Opin Neurol, 19(3), 294-298.

Sarchielli, P., Alberti, A., Floridi, A., & Gallai, V. (2001). Levels of nerve growth factor in cerebrospinal fluid of chronic daily headache patients. Neurology, 57(1), 132-134.

Schur, E. A., Noonan, C., Buchwald, D., Goldberg, J., & Afari, N. (2009). A Twin Study of Depression and Migraine: Evidence for a Shared Genetic Vulnerability. Headache, 49(10), 1493-1502.

Stam, A. H., de Vries, B., Janssens, A. C., Vanmolkot, K. R., Aulchenko, Y. S., Henneman, P., et al. (2010). Shared genetic factors in migraine and depression. Evidence from a genetic isolate. Neurology, 74(4), 288-294.

Stewart, W. F., Lipton, R. B., Celentano, D. D., & Reed, M. L. (1992). Prevalence of migraine headache in the United States. Relation to age, income, race, and other sociodemographic factors. Jama, 267(1), 64-69.

Tomkins, G. E., Jackson, J. L., O'Malley, P. G., Balden, E., & Santoro, J. E. (2001). Treatment of chronic headache with antidepressants: a meta-analysis. Am J Med, 111(1), 54-63.

Van den Maagdenberg, A. M., Haan, J., Terwindt, G. M., & Ferrari, M. D. (2007). Migraine: gene mutations and functional consequences. Curr Opin Neurol, 20(3), 299-305.

Visscher, P. M. (2008). Sizing up human height variation. Nat Genet, 40(5), 489-490.

Wessman, M., Kallela, M., Kaunisto, M. A., Marttila, P., Sobel, E., Hartiala, J., et al. (2002). A susceptibility locus for migraine with aura, on chromosome 4q24. Am J Hum Genet, 70(3), 652-662.

Zwart, J. A., Dyb, G., Hagen, K., Odegard, K. J., Dahl, A. A., Bovim, G., et al. (2003). Depression and anxiety disorders associated with headache frequency. The Nord-Trondelag Health Study. Eur J Neurol, 10(2), 147-152.

Nederlandse Samenvatting

Migraine is een ernstige en veelvoorkomende hoofdpijnaandoening met grote impact op het leven van patiënten en hun familieleden. Een migraineaanval wordt gekenmerkt door matige tot ernstige hoofdpijn, vaak bonkend of kloppend en gelokaliseerd aan één kant van het hoofd. De hoofdpijn wordt vaak verergerd door fysieke activiteit. Tevens kan migraine gepaard gaan met overgevoeligheid voor licht en geluid, en misselijkheid en/of braken.

De twee meest voorkomende typen migraine zijn migraine met aura en migraine zonder aura. Het aura is een plaatselijke neurologische stoornis, die aan het begin van de aanval optreedt en van voorbijgaande aard is. Meestal gaat het om visuele stoornissen, zoals het uitvallen van een deel van het gezichtsveld (blinde vlekken), zigzagpatronen of lichtflitsen. Wat ook veel voorkomt zijn bijvoorbeeld tintelingen en een doof gevoel in de ledematen of spraakproblemen.

Hoewel we een redelijk beeld hebben van de mechanismen die de hoofdpijn en overige symptomen van migraine verklaren is er over de oorzaak nog relatief weinig bekend. Het doel van dit proefschrift was om meer te weten te komen over de erfelijkheid van migraine, over het verband tussen migraine en depressie, en ten slotte om met koppelings- en associatie onderzoek de genen te lokaliseren en identificeren die migraine veroorzaken.

Het proefschrift begint in hoofdstuk 1 met een overzicht van de literatuur over de epidemiologie en pathofysiologie van migraine. Het beschrijft hoe migraine wordt gediagnosticeerd, wat we weten over de onderliggende mechanismen, en over het verband tussen migraine en diverse psychiatrische en ook niet-psychiatrische aandoeningen. Ook wordt er een overzicht gegeven van wat er bekend is over de genetica van migraine. Wat we hiervan weten is voornamelijk afkomstig van een ernstige en zeldzame vorm van migraine, genaamd familiare hemiplegische migraine (FHM). Er zijn mutaties in een drietal genen geïdentificeerd die deze aandoening kunnen veroorzaken. Studies naar 'gewone' migraine hebben nog niet geleid tot de identificatie van risicogenen.

In hoofdstuk 2 wordt een overzicht gegeven van methoden die veel worden gebruikt in de genetische epidemiologie, en ook in dit proefschrift. Om de erfelijkheid van een aandoening te bepalen worden veelal tweelingstudies gebruikt. Linkage ('koppelingsonderzoek') wordt gebruikt om gebieden in het genoom, die betrokken zijn bij een aandoening te lokaliseren met behulp van familiedata en genetische markers zoals microsatelliet-data. Associatiestudies (doorgaans in ongerelateerde personen) worden gebruikt om te bepalen welke

genetische varianten (single nucleotide polymorphisms; SNPs) betrokken zijn bij een aandoening zoals migraine.

Hoofdstuk 3 en 4 beschrijven de datacollectie die voorafging aan de analyses. Dit onderzoek is voornamelijk gebaseerd op data van het Nederlands Tweelingenregister (NTR). Door middel van vragenlijstonderzoek verzamelt het NTR data over gezondheid, persoonlijkheid en leefgewoonten bij tweelingen en hun familieleden. In 2002 en 2004 zijn vragenlijsten verstuurd waarin uitgebreid werd gevraagd naar het voorkomen van hoofdpijn en specifiek naar symptomen van migraine. Hoofdstuk 3 beschrijft onder andere de samples die gebruikt zijn in dit proefschrift en de vragenlijst waarmee migraine werd gemeten. In hoofdstuk 4 wordt onderzocht of er verschillen zijn tussen mensen die wel en niet meededen aan het onderzoek. Hierin werd gevonden dat er geen verschil in migraineprevalentie was tussen mensen die wel en niet meedoen aan het vragenlijstonderzoek van het NTR.

In hoofdstukken 5, 6 en 7 werd gekeken naar de fenotypische kenmerken van migraine, gebaseerd op de symptomen die zijn opgenomen in de officiële diagnostische criteria van de International Headache Society. Ook werd de comorbiditeit van migraine en depressie onderzocht, en de invloed van depressie op de erfelijkheid van migraine. De data hiervoor waren grotendeels afkomstig uit het vragenlijstonderzoek van het NTR; aanvullende data over depressie kwamen uit een grootschalig Nederlands depressieonderzoek (de Nederlandse Studie naar Depressie en Angst; NESDA).

In hoofdstuk 5 werd latente klassen analyse gebruikt om te onderzoeken of er verschillende subtypen migraine bestaan. Een belangrijke vraag hierbij was of er aparte subtypes bestonden die overeenkwamen met de classificatie van migraine met aura (MA) en migraine zonder aura (MO). Volgens de officiële diagnostische criteria zijn MO en MA twee verschillende typen migraine, maar is daar ook empirisch gezien evidentie voor? Dit hebben we onderzocht door mensen te groeperen aan de hand van het patroon van symptomen dat ze rapporteerden. Uit de resultaten bleek dat patiënten voornamelijk gegroepeerd werden op basis van de ernst van hun migraine. Er waren weinig kwalitatieve verschillen, behalve dat in de ernstigste groep relatief vaker overgevoeligheid voor licht en geluid, misselijkheid/braken en aura voorkwam. Er was echter geen evidentie voor een subtype migraine dat specifiek gekenmerkt werd door aura. Zelfs in de groep met de ernstigste migraine had slechts ongeveer de helft van de mensen aurasymptomen. De erfelijkheid van migraine op basis van deze empirische classificatie werd geschat op 50%. Op basis van de officiële definitie was dat 49%. Samenvattend is er, op grond van de resultaten van de latente

klassen analyse, geen evidentie voor een specifiek subtype van migraine met aura.

Van depressieve patiënten weten we dat ze bovengemiddeld vaak aan migraine lijden. In hoofdstuk 6 werd onderzocht of depressieve patiënten aan dezelfde vorm van migraine lijden als niet-depressieve mensen, of dat er een subtype van migraine is dat geassocieerd is met depressie. Dit werd onderzocht met behulp van latente klassen analyse. De symptoomprofielen van depressieve patiënten en controles werden met elkaar vergeleken om te kijken of er kwalitatieve verschillen te zien waren. Hoewel de prevalentie van migraine, zoals verwacht, duidelijk hoger was in depressieve patiënten, werden er geen grote kwalitatieve verschillen gevonden. Depressieve patiënten rapporteerden iets vaker dat de hoofdpijn verergerde bij fysieke activiteit, en ook hadden ze iets vaker aurasymptomen. Verder waren de symptoomprofielen vrijwel gelijk. Het lijkt er dus op dat depressieve patiënten en controles aan hetzelfde type migraine lijden. Overigens betekent dit niet dat we mogen aannemen dat de oorzaak van migraine ook hetzelfde is in beide groepen.

In hoofdstuk 7 werd gezocht naar een verklaring voor de comorbiditeit van migraine met angst en depressie. Zou het zo kunnen zijn dat dezelfde set van genen beide aandoeningen beïnvloedt? Dit is goed mogelijk aangezien beide aandoeningen deels genetisch zijn (de erfelijkheid van angst/depressie was in onze studie ongeveer 55%). Verder hebben we getest of de erfelijkheid van migraine verschilt in mensen met een hoge en lage score op een maat voor angst en depressie. Tenslotte werd onderzocht of van het verband tussen migraine en angst/depressie mogelijk verklaard zou kunnen worden door een causaal verband (de ene aandoening leidt tot een verhoogde kans op de andere aandoening). De resultaten lieten zien dat migraine en angst/depressie voor een deel inderdaad door dezelfde genen worden beïnvloed. Verder bleek dat de erfelijkheid van migraine het hoogst was in mensen die laag scoorden op angst en depressie. Tot slot bleek uit een analyse van discordante tweelingparen dat het verband tussen de twee aandoeningen zich niet enkel laat verklaren doordat dezelfde genen meerdere aandoeningen beïnvloeden, maar dat er waarschijnlijk een causaal verband is. Mogelijk maken depressie en migraine bij sommige patiënten deel uit van een syndroom.

Hoofdstuk 8 en 9 richten zich op onderzoek naar welke genen migraine veroorzaken. Dit werd onderzocht met behulp van twee verschillende methoden: koppelingsonderzoek (linkage) en genoom-brede associatie (genome-wide association, afgekort tot GWA). In hoofdstuk 8 worden de resultaten van een linkage-studie beschreven. Deze studie bracht verschillende

regio's aan het licht die mogelijk betrokken zijn bij migraine, bijvoorbeeld op chromosoom 1q23, 13q32 en 20p12. Onze bevinding op chromosoom 1 lag zeer dicht bij het *ATP1A2* gen dat ook betrokken is bij FHM. Dit duidt erop dat dit FHM-gen mogelijk ook een rol speelt bij gewone migraine. Ook werden een aantal eerdere bevindingen gerepliceerd, zoals een locus op chromosoom 5q21, eerder beschreven in een Australische linkage-studie, en een gebied op chromosoom 10 wat gevonden werd in Australische en Finse linkage-studies. De bevinding op chromosoom 20 werd onlangs gerepliceerd in een linkage studie naar migraine in combinatie met bipolaire stoornis. Mogelijk is dit gebied bij beide aandoeningen betrokken.

In hoofdstuk 9 wordt een meta-analyse beschreven van GWA-studies in 6 verschillende Europese samples. Hierin vinden we verdere aanwijzingen voor de betrokkenheid van *ATP1A2* bij gewone migraine. Het beste resultaat werd gevonden in het *NGFR* gen (de nerve growth factor receptor). Dit is een uitermate plausibel kandidaat-gen voor migraine, aangezien het een belangrijke rol speelt in pijnperceptie. De NGF receptor komt onder andere voor in het trigeminale ganglion, wat een cruciale rol speelt bij pijnwaarneming en sensitisatie tijdens een migraine-aanval.

Verdere technologische ontwikkelingen op het gebied van genotyperen, het beschikbaar komen van grotere groepen patiënten met genoom-brede SNP data, verbeteringen in de statistische methoden om zulke gegevens te analyseren en follow-up methoden van de beste SNP resultaten zullen in de toekomst waarschijnlijk tot de ontdekking van meer kandidaat-genen voor migraine leiden. Het succes van de zoektocht naar migrainegenen is echter niet alleen afhankelijk van technologie. Een andere belangrijke factor is hoe we migraine meten. Niet alleen het diagnosticeren van de migraine zelf is van groot belang, maar ook het in kaart brengen van de aanwezigheid van comorbide aandoeningen zoals angst en depressie. In sommige gevallen kan de aanwezigheid van andere aandoeningen iets zeggen over de oorzaak van de migraine, bijvoorbeeld bij bepaalde neurologische aandoeningen. Het is goed voorstelbaar dat dit ook het geval is bij het samen voorkomen van migraine en psychiatrische stoornissen. Als we hier rekening mee houden kan dit het identificeren van de betrokken genen ook makkelijker maken. Het systematisch in kaart brengen en onderzoeken van het verband tussen migraine en comorbide aandoeningen kan ons mogelijk veel leren over het ontstaan ervan.

Als de genen die betrokken zijn bij migraine gevonden worden, zullen er functionele studies gedaan moeten worden om uit te wijzen wat de

achterliggende mechanismen zijn: hoe zorgt een bepaalde genetische variant ervoor dat er migraine ontstaat? Meer inzicht in deze mechanismen zal op den duur hopelijk leiden tot de ontwikkeling van verbeterde behandeling van migraine.