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Genetic epidemiological approaches in complex neurological disorders

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Citation

Hottenga, J. J. (2005, November 10). *Genetic epidemiological approaches in complex neurological disorders*. Retrieved from https://hdl.handle.net/1887/4343

Version:	Corrected Publisher’s Version
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Note: To cite this publication please use the final published version (if applicable).

CHAPTER 1

Introduction

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Complex neurological disorders

The prevalence of various complex neurological disorders, like migraine and Alzheimer's disease, is high in the general population[1-4]. Although complex neurological disorders are different in pathology and clinical manifestation, the impact on the quality of life of patients and the socio-economic level of the population is undoubtedly substantial[5-8]. The quality of life is reduced by the, often progressive, nature of the disorders and the lack of adequate treatment. Society and economy are burdened by costs of treatment, hospitalizations and loss of active working days of affected people. Findings that may help to reduce this impact are therefore of high importance. However, for most neurological disorders the pathophysiology, biochemical pathways and causative factors are complex and still largely unknown.

Neurological disorders are complex in various ways. A simple limitation is that brain tissue is difficult to study and research questions often have to be answered by other study designs. Another more important complexity is the often multifactorial nature of these disorders[9]. Multiple risk factors, environmental as well as genetic, contribute to the disorder individually or by means of interaction. Each independent risk factor increases the susceptibility, but not all risk factors are required to cause the trait. The multifactorial aspect also applies to genetic risk factors. As a result, the disorders cluster in families, but contrary to Mendelian monogenic disorders, there is often no clear mode of inheritance[10].

Gene identification in complex neurological disorders

In many complex neurological disorders a substantial part of the aetiology can be ascribed to genetic factors. In migraine, epilepsy and Alzheimer for example, the estimated heritability or proportion of variance explained by genetic factors, is ~46%, ~70% and ~48%, respectively[11-13]. Identification of these genetic factors is frequently an initial key step in understanding the

pathophysiology. Positional cloning is an often used method to identify genes. It involves essentially two steps, namely the identification of the region on the genome involved in the disorder (locus mapping through genome scans), followed by identification of the causative gene. In a genome scan, a narrow grid of markers evenly spaced over the genome is tested. For this purpose highly polymorphic microsatellite - repeat markers are used that have between two and thirty repeats (alleles), each consisting of two to six nucleotides. The marker alleles are subsequently correlated with the segregation of the disorder, leading to the identification of the genomic region(s) harboring the disease gene(s). Next, candidate genes in these regions are prioritized for further analysis. For Mendelian, monogenic, disorders, candidate genes are analyzed (for instance by sequencing) to identify high-impact mutations (missense, non-sense, deletions, insertions etc.). In the case of complex traits, one has to identify low-impact variants (polymorphisms). To this end, a denser grid of single nucleotide polymorphism (SNP) markers (bi-allelic) can be tested by association studies, followed by functional validation such as analysis of changed expression of the causative gene in affected individuals.

An alternative is the candidate gene approach; directly selecting candidate genes without prior genome scan experiments. The selection of a candidate gene is based on pathological, biochemical or molecular knowledge of the disorder. The candidate gene approach thus provides an opportunity to quickly assess the involvement of genes. This is useful to exclude known genes or to confirm / replicate findings of other studies. Nowadays, candidate genes can also come from for instance transcriptomics and proteomics studies.

In this thesis the main focus will be on the use of techniques involved in positional cloning. In recent years, the use of positional cloning has exponentially increased the number of genes known to be involved in human monogenic diseases[14]. For complex genetic traits including many neurological disorders, the successes have been more limited.

Problems in gene identification of complex traits

Trait definition

In neurological disorders there is often a lack of biological markers and diagnosis is based mainly on the presence of clinical symptoms. Although international diagnostic criteria for many disorders have been established, problems remain with their implementation in genetic studies[15-18]. There can be large variation in the expression of a disorder in patients of a family, making the inclusion or exclusion of these individuals as being affected for the study difficult. Diagnostic criteria such as 'severity' can be interpreted differently by patients and physicians. There can also be heterogeneity when patients have different subsets and/or frequency of clinical symptoms. For example, the presence and frequency of vomiting and phonophobia in migraine patients can be different[16]. Additional variation in phenotype can be caused by co-morbidity and clinical overlap of symptoms. In Alzheimer's disease for example, there is a large overlap with other dementias like vascular dementia and Parkinson[19,20]. Patients with epilepsy can sometimes be characterized with more than one syndrome. Therefore, the definition of neurological traits as phenotypes to be analyzed in genetic studies is in many cases not optimal.

Genetic Heterogeneity

Genetic heterogeneity is a major reason why neurological disorders are complex[9,10,14]. In linkage analysis genetic heterogeneity is often categorized in allelic - and locus heterogeneity. Allelic heterogeneity refers to the situation that multiple alleles of a single gene are related to an increased risk of the disorder, whereas locus heterogeneity refers to multiple genes involved in the disorder. Genetic heterogeneity may obscure the mode of inheritance, when autosomal recessive (2 risk alleles required for a trait) -, dominant (a single risk allele sufficient for a trait) - as well as chromosome X linked genes are involved. More important, in gene-mapping studies, affected families or persons not sharing the same genetic variant (phenocopy) contribute

negatively to the study outcome. Across populations, heterogeneity will cause difficulties for study replication, as it remains a question whether the genes found in one population are also risk factors in another[21]. For complex diseases, failure of detecting or exclusion of a specific risk factor in a given family does not mean that it is not a risk factor in other families. Heterogeneity has been reported for many traits including rare Mendelian disorders. An example is familial hemiplegic migraine (FHM), in which at least three genes lead to the development of this trait[22-25].

Interactions

Frequently risk alleles of multiple genes are required to cause a complex disorder, therefore, gene-gene interactions should be taken into account. For example, the Apolipoprotein ε4 allele (APOE*4) is an established risk factor for Alzheimer's disease, which is currently frequently included as a covariate in association studies[26,27]. Likewise, environmental factors can alter the effects of genes; gene-environment interaction. Without interactions, the risk of genes is considered to be additive; the risk for a subsequent harmful allele is increasing the total risk of the disorder independent of other risk factors. However, the risk of the allele can also be related to the presence of other risk factors, where the risk is much higher or lower than the expected risk based on the individual risk factors (non-linear effects, interaction). In a more extreme case, a disorder may be present only when multiple risk factors are present simultaneously (gene-epistasis). Currently a few statistical linkage methods can be employed to take multiple genes or environmental covariates into account and these are infrequently applied[28-30]. The sample sizes required for detecting interactions are substantial and may become prohibitive[31]. Genes interacting with the environment may be detected in specific populations only. Like with heterogeneity, this hampers study replication, which is considered good evidence for true causality[10,32,33].

Methods for identifying complex disease genes

As mentioned in the previous section, the exact strategy to identify the causative gene defect in monogenic disorders may differ from that in complex traits, but both strategies make use of positional cloning of genes (gene-mapping) and the analysis of candidate genes. For gene-mapping in complex diseases, linkage and sib-pair analysis are more suited, while association studies and transmission disequilibrium tests are more frequently employed to study candidate genes. Furthermore, the methods can be employed to study both dichotomous traits as well as quantitative trait loci (QTLs) in which the trait is a continuous variable[34].

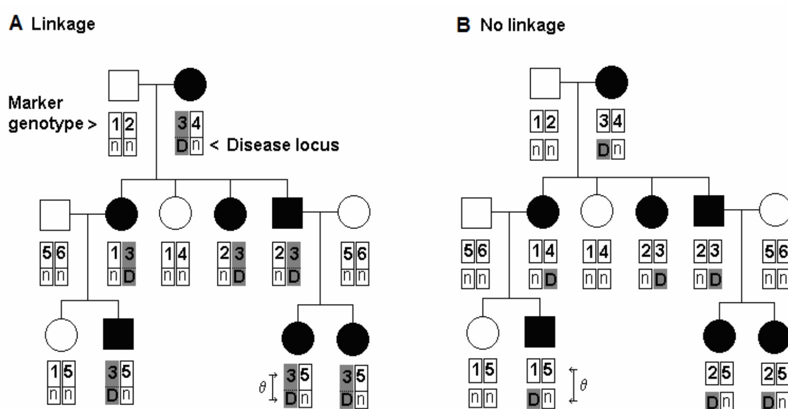
Linkage studies in extended families

Hallmark of linkage analysis is a process called recombination. During meiosis, recombination occurs between homologous chromosomes in either parent leading to two new hybrid chromosomes (gametes) that are transmitted to the offspring[35]. In case one of the parents carries a risk gene, only a part of the chromosome and marker alleles close to this gene will remain 'linked' to the gene over several generations (linkage disequilibrium). When the distance on the chromosome between the risk gene and the tested marker increases, the probability of recombination increases as well, and linkage disequilibrium diminishes. Testing for linkage in a family means that one evaluates to what extend the disorder co-segregates with a tested marker allele (single point analysis) or multiple marker haplotype (multipoint analysis)[36]. Under the null hypothesis the maximum likelihood of the observed marker data assuming no linkage with the disorder is calculated (recombination probability $\theta = 0.50$) (figure 1). This likelihood is subsequently compared with the maximum likelihood under the assumption that the given marker data (an allele or haplotype) is linked to the disorder ($\theta < 0.50$). The 10-log likelihood ratio, or LOD score, is calculated to indicate if the alternative hypothesis (i.e. the presence of linkage) is better or worse than the hypothesis assuming no

linkage. A LOD score above 3.3 is generally considered significant evidence for linkage in genome scans[33]. In addition to testing single families, the same approach can be applied to test multiple families at once. The marker of choice for linkage analysis is often the microsatellite marker as it has the highest informativeness (heterozygosity) in the parental transmission of alleles.

Figure 1

The principle of linkage presented in a single family.



To test the hypothesis of linkage the segregation of the disease locus *D* is correlated with genotypes of a multi- allelic marker. The likelihood of the family data is maximized for the recombination probability θ. In the linked family the dominant disorder is fully co-segregating with maternal allele 3 (figure 1A). The maximum likelihood is found at $\theta = 0.00$ as no recombinations were observed between allele 3 and disease locus *D*. In the other family there is no linkage between any of the marker alleles and the disorder (figure 1B). There is no consistent co-segregation and several recombinations should have taken place in order to maintain linkage evidence. The maximum likelihood is found at $\theta = 0.50$ equaling the null hypothesis of no linkage.

Statistical analysis for linkage can be done with parametric (model-based) or non-parametric (model-free) methods[37]. In the model-based approach several parameters have to be specified in order to calculate the maximum likelihood for the linkage statistic[36,37]. These are the gene frequency of the disorder, the phenocopy probability and the probabilities of being affected while carrying

one - or two copies of the risk allele (penetrances). With the parameters, the model and mode of inheritance are fixed. The correctness of this model may influence the study outcome[38-40]. Studies have shown that the effect of wrong specification of the linkage model in single point analysis is generally low, except for the mode of inheritance[41-43]. Segregation analysis can be used to find the best fitting mode of inheritance and model parameters[44-46]. For parametric linkage analysis several programs, such as FASTLINK or MENDEL are available[47-49]. In the model-free analysis the likelihood ratio is based only on the sharing of alleles between affected and non-affected individuals. These are compared with the expected random segregation of alleles. As a result the non-parametric approach is less susceptible to spurious results due to wrong specification of the model. The cost of using model-free methods is often a reduction in power to detect linkage as compared to a *correctly* specified model-based method[42,43]. Non-parametric linkage for dichotomous traits or QTLs can be tested with programs like MENDEL, GENEHUNTER, MERLIN and SOLAR[49-53].

Linkage analysis is sensitive to genetic heterogeneity[54]. A way to reduce this heterogeneity is to select a more homogenous sample of families. High-impact risk factors do exist for complex traits; there are families in which the disorder and risk alleles seem to follow a Mendelian pattern of inheritance (i.e. with an almost one-to-one correlation between the gene and the disorder). Often the phenotype of patients within these families is more consistent; symptoms may have an earlier age at onset or additional characteristics may be present[16,55,56]. Selecting these families, thereby reducing the heterogeneity, and applying linkage analysis has often been a successful first step into the molecular biology of complex neurological disorders[55,57,58]. Another approach to analyze a larger sample of families, is to take the heterogeneity of loci into account with programs like HOMOG, or to analyze the data using liability classes[36,59]. Finally, locus homogeneity of studies may also be improved by selecting a sample from more homogenous isolated populations[60,61].

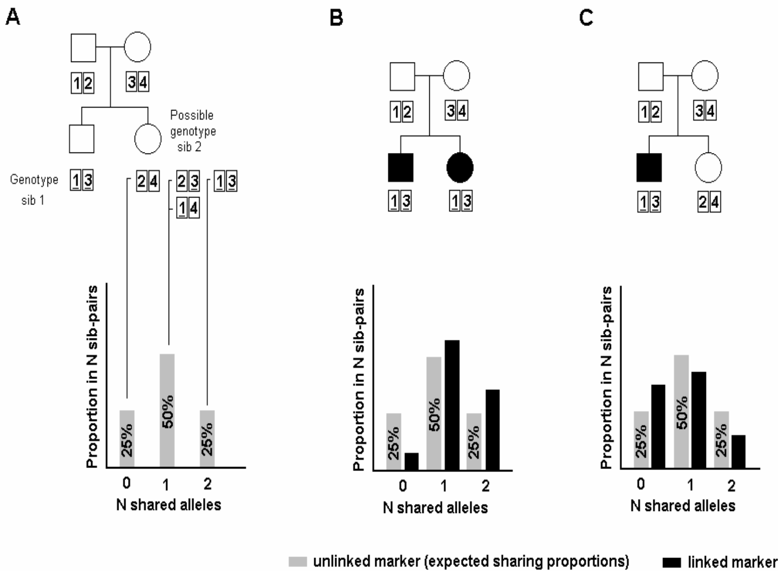
Sib-pair studies

In sib-pair studies the sharing of alleles between two siblings is studied in relation to the phenotype / disorder[62]. At a given locus, each sibling receives two of the four alleles that can be transmitted by the parents. As a result, a sib-pair will share 0, 1 or 2 alleles for a locus as shown in figure 2. The sharing is called identity-by-descent (IBD) in case the genotypes of the parents are discrete and the alleles that the siblings share can be scored exactly. In case the parents' genotypes are ambiguous and the exact sharing of the alleles (phase) cannot be determined, the sharing is called identity-by-state (IBS). With the IBS/IBD status of the individual pairs, a summation of the probabilities sharing 0, 1 or 2 alleles for all pairs can be calculated. For a random marker not related to the disorder these expected sharing probabilities are 25%, 50% and 25% (figure 2A). When linkage is present between the marker and the disorder, excess sharing of alleles is expected in affected (concordant) sib-pairs (figure 2B). A Z-score statistic, comparing the expected with the observed sharing probabilities for a marker, can be used as a test for linkage. Since no prior genetic model for the allele segregation needs to be assumed, sib-pair analysis is a non-parametric test for linkage. The marker of choice for sib-pair analysis is the microsatellite repeat marker, as multiple alleles give the most information about the parental transmission.

In addition to affected sib-pair analysis, other types of sib-pair analyses are possible. One is testing discordant sib-pairs; where only one sib is affected, in which the assumption is made that sib-pairs share less alleles than expected (figure 2C)[63,64]. Also QTLs can be studied where the trait variance between sibs is correlated with the number of shared alleles[65-67]. Affected sib-pair -, discordant sib-pair - and QTL analysis are implemented in various software packages like MAPMAKER SIBS, GENEHUNTER, MERLIN, MENDEL or SOLAR[49-53,62]. These will calculate the IBD probabilities as well as the various LOD score statistics.

Figure 2

The principle and expected sharing proportions of alleles in sib-pairs for a concordant - and discordant sib-pair study design given an unlinked and linked marker for a (dominant) disorder.



For N sib-pairs the expected proportions of 0, 1 or 2 alleles are 25%, 50% and 25% when there is no linkage, represented by the grey bars (figure 2A). A hypothetical marker linked to the disorder is shown in the black bars. In case of analyzing a sample of concordant sib-pairs this marker will show increased sharing proportions of 1 and 2 alleles (figure 2B). When analyzing a sample of discordant sib-pairs the marker will show a decreased sharing of 1 and 2 alleles (figure 2C). The heights of the black bars are potential outcomes of such analyses.

The sib-pair design is one of the most robust designs for gene mapping. Unlike association studies this design is not affected by confounding of population stratification. Also, as compared to the parametric or model-based linkage methods in extended families, they are less susceptible to large effects of heterogeneity, non-penetrance and phenocopies in single families[10]. Unfortunately, the power to detect loci in complex disorders for this design is often low[42,68]. When a locus is detected, the shared region on the genome

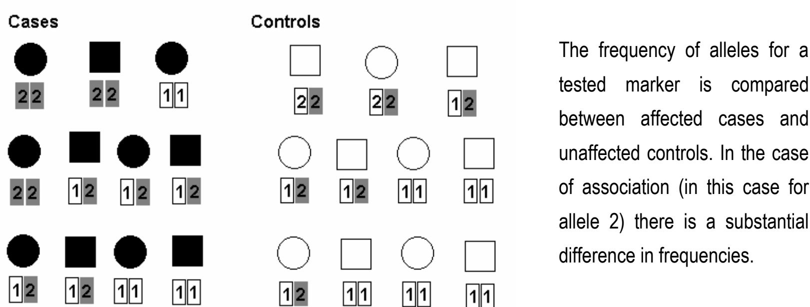
between two sibs is generally much larger compared to family or association studies hampering subsequent gene identification[69].

Association studies

In an association study the frequency of (a) specific marker allele(s) is compared between a group of unrelated patients (cases) and a group of unaffected individuals (controls) (figure 3). The assumption made is that the studied allele encodes a variant that increases the risk for the disorder. Compared to family-based designs, association studies have more power to detect genes with a relatively small influence on the disorder[68]. The use of SNP markers is preferred, as the power to detect gene effects is optimal for bi-allelic markers with a high gene frequency and the mutation rate of SNPs is generally lower[70,71]. Association studies can be applied to test single candidate genes and for genome scans testing up to 100 000 SNPs. Currently, the application of the association study for genome scans is still limited, however with the maturing of rapid and cheap SNP genotyping technology, the introduction of the HapMap project and advancing statistical methods this is about to change[72-74].

Figure 3

The principle of an association study.



The frequency of alleles for a tested marker is compared between affected cases and unaffected controls. In the case of association (in this case for allele 2) there is a substantial difference in frequencies.

Compared to family linkage studies the collection of data for association studies is simple and cost-effective. For late-onset disorders, like Alzheimer's disease, it may be the favored method of choice because relatives like parents and siblings are often not available anymore. Selection of cases and controls can be done using preferably large epidemiological studies[75]. Cases and controls are preferably matched for age, gender, population origin and other risk factors to control for confounding variables. For the statistical analysis of association studies many classic epidemiological methods can be applied[76]. These methods include the Pearson χ^2 statistic, odds ratio and relative risk analysis, logistic regression, survival analysis and ANOVA tests for QTLs. Before commencing on testing differences in allele frequencies, it is advisable to test for Hardy-Weinberg equilibrium (HWE) in cases and controls[77]. This can exclude large influences of selection bias, population stratification and genotyping errors.

Association between a marker and a disease will be found in four situations. In the first 'lucky' situation the tested marker is directly the functional polymorphism that causes the disorder. In this case, follow-up studies should aim at studying the gene effects preferably using other methods in independent study samples[32,75]. In the second situation the marker is in close linkage disequilibrium with the gene-variant that influences the disorder. Recent studies have shown that the linkage disequilibrium between several SNPs in candidate genes is variable and may extend to only a few kilobases[78,79]. The expected shared genomic regions between cases are likely to be very small[69]. Testing other SNPs in the same gene and studying for instance gene expression is therefore required for identification of the functional variant(s). The third reason for finding a positive association is confounding. A frequently mentioned problem is population stratification[10,80,81]. Here, the cases and controls are ascertained from two populations, which differ in gene frequencies and disease risk. In the case and control groups the representation of these populations is therefore unequal, and tested markers that have a

different gene frequency in both populations will be associated with the disorder. The fourth reason for finding association is that the result is a statistical false-positive[75,81-83]. Given a significance level of 0.05, which is frequently used for association studies, the probability of false-positive results is substantial. Given that up to 15 million variants and about 30 000 genes are present in the human genome, the probability of selecting the right SNP(s) *a priori* is extremely small[14,70,84,85] . This problem may be reduced by careful selection of candidate genes, but a recent review showed that many candidate gene associations may be false-positives[70].

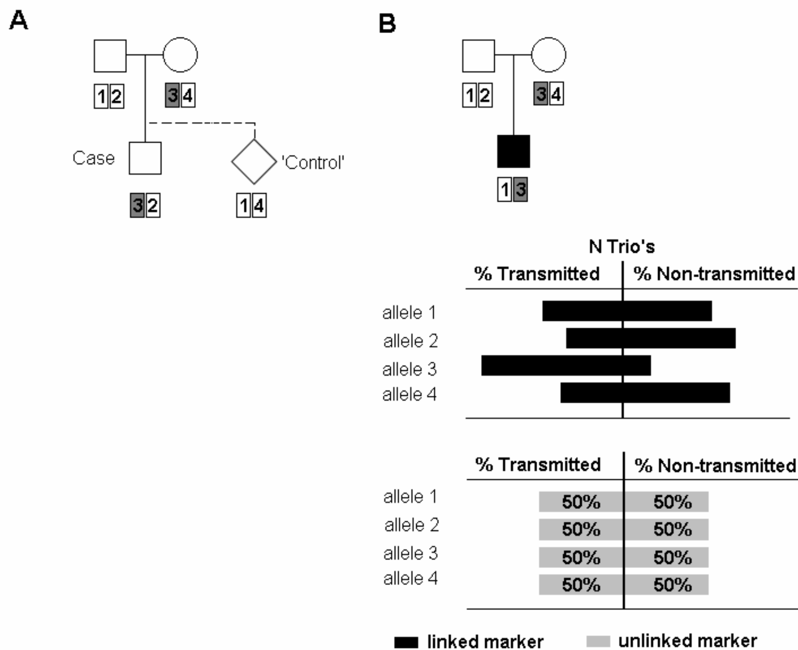
Several suggestions have been made to improve association study designs. These include testing for population stratification and other possible confounders, and to increase the significance level for reporting associations[14,75,86,87]. Also the study sample sizes, given the relative risks found for various associations, should be sufficiently high[70,88]. Taking into account the restrictions of the design, the ease of data collection, epidemiological analysis and the high power to pick up genes with relatively small effect size make the design a useful tool to study complex neurological disorders.

Family-based association studies

Family-based association studies are good alternatives for the straightforward case-control design to maintain the flexibility of the case-control approach without the confounding of population stratification. Several methods have been developed. The first was the haplotype relative risk (HRR) method[89,90]. Here, the two parents of a patient are also genotyped and the transmission of alleles to the case is compared with a pseudo-control assuming to have the alleles not transmitted to the case (diamond in figure 4A). Although this approach reduced the effects of population stratification, it could not eliminate them completely[91]. Another approach to the population stratification problem was the transmission disequilibrium test (TDT) (figure 4B)[92].

Figure 4

The Haplotype Relative Risk method and Transmission Disequilibrium Test principles.



A) In the haplotype relative risk method the non-transmitted alleles of the heterozygous parents are considered to be the genotype of the putative control (diamond). Standard analysis of association can subsequently be applied to test the hypothesis of association. B) The TDT approach compares the transmission of alleles from parents to offspring with the expected random Mendelian transmission (grey bars). In case of association the transmission of a specific allele (3) is increased while others are decreased (2,4), as shown for a hypothetical linked marker in the black bars.

The rationale behind the TDT is that the alleles are assumed to be transmitted randomly from parents to offspring. The TDT compares the number of times each allele was transmitted or not transmitted to an affected offspring by means of a χ^2 statistic. In case a marker allele is related to the studied disorder, the transmission of this allele will be increased in cases. The TDT test can be applied to study association of alleles as well as linkage, and is therefore useful for fine mapping of disease genes. For association testing, only one trio

should be taken per family if the original TDT statistic is applied, because cases are otherwise not independent[91,93]. For testing linkage, extended families can be tested as well.

Several extensions to the TDT have been proposed over the recent years. One was to use markers with multiple alleles accounting for the loss of information caused by parental homozygosity, while maintaining the advantage of correction for population stratification[94-97]. Furthermore, the use of haplotypes / multiple markers with - or without known haplotype data of the parents has been proposed[94,97]. Other adjustments were made by various authors to incorporate QTLs or covariates like age and sex[98-101]. However, most extensions were made to account for the TDT requirement to have both parents available, a substantial problem in late-onset disorders. The use of other family members, most notably siblings, was implemented in various tests to account for missing parent data[102-104]. Family members were used both for reconstruction of parental genotypes/haplotypes, as well as to test the transmission over different family members[94,102,105-107]. With the inclusion of family members, the use of the affection status of these members was also considered, increasing the sample size and information per family. As previously mentioned the association of a marker then becomes dependent on the number of family members present in the sample. Various statistics handling this problem have been developed and this has led to the current situation in which these methods have become a hybrid analysis of association, sib-pair and /or linkage that can be applied to numerous family constellations[93,108-111].

Genetics of neurological disorders studied in this thesis

In this thesis genetic epidemiological methods were applied to various neurological disorders. Here, short summaries of the disorders and their main genetic findings are presented in order to provide some background of their complexity.

Alzheimer's disease

Alzheimer's disease (AD) is characterized by a gradual onset of decline of memory and problems in at least one other area of cognition. Additional characteristics are a gradually progressive course of the disorder with a preserved level of consciousness. AD is a frequent late onset disorder, going from a male and female prevalence of 1.2% in people between 65 and 69 years old, to a prevalence of 33% in people aged up to 90 and older[3,4,112,113]. Diagnosis of AD is made based on extensive clinical anamnesis following the NINCDS-ADRDA criteria[17]. The diagnosis can sometimes be ambiguous, as both vascular dementia and Parkinson's disease have a large clinical overlap with AD[19,20]. The pathology of AD shows extra cellular plaques mainly composed of amyloid β peptide and intracellular neurofibrillary tangles containing hyperphosphorylated protein[114]. AD is also heterogeneous in age at onset and is often divided into groups with early-onset AD and late-onset AD for research and clinical purposes. The exact age which distincts early- from late-onset AD is fixed at 65 years, but remains a matter of discussion.

Particularly for early-onset families, but also for late-onset AD, twin and familial studies have shown that there is a strong heritable component for AD[13,115,116]. Exactly how much of the pathology of Alzheimer's disease can be explained by genetic factors is somewhat ambiguous; heritability estimates range from 29 to 78% [115]. This is mainly due to the variable late onset of the disorder, since persons might still become affected or are censored because of mortality. Segregation analysis of early-onset families has shown that there is not only a large single genetic component as the multifactorial model fits optimally[116].

For the early-onset Mendelian forms of AD several genes are known. The first gene that was found using linkage analysis in early-onset AD families was the transmembrane amyloid precursor protein (APP) on chromosome 21q21 [117,118]. Subsequently, mutations in two other genes, Presenilin-1 and Presenilin-2

(PSEN1 and PSEN2), were identified on chromosomes 14q24 and 1q42, respectively[119-121]. Although mutations in these three genes are frequently found in families with AD, these are accounting for only a few percent of the total number of AD cases in the general population. Another gene variant APOE*4, is accountable for a more substantial part of the population AD cases. The APOE*4 allele is an established risk factor for AD and is one of the most replicated associations studied[26,70]. New loci for late-onset AD have been found on chromosomes 10p11.23-q22.3, 12p12.3-q13.13 and 20p11.23-q12, but no consistent results of mutations related to AD have been found in these areas[122-124]. Gene-gene interaction and gene-environment interaction, especially with APOE*4 are frequently studied[125-127]. The interactions as well as the large heterogeneity make AD a complex disorder to study.

Migraine

Migraine is a common neurovascular disorder manifested by attacks of severe disabling headache. Anyone may have a migraine attack sometimes but the frequency of the attacks makes the disorder. The lifetime prevalence of migraine is up to 6% of men and 18% of women in the general population[1,2]. Diagnosis is made on the basis of a patient's history and is categorized in attack types using standardized diagnostic criteria defined by the International Headache Society (IHS)[16]. Attacks of migraine without aura (MO) are characterized by severe, often unilateral, throbbing headache that is aggravated by physical activity and is accompanied by other disabling neurological symptoms like vomiting, nausea, photophobia and/or phonophobia. One third of the migraine patients also develops visual aura symptoms, which are preceding or accompanying the headache; migraine with aura (MA).

Migraine is a complex disorder in which both environmental as well as genetic factors are involved[128,129]. The estimated heritability for the common types of migraine is 46% [11]. In addition, migraine can also be a part of autosomal dominant cerebrovascular syndromes, such as cerebral autosomal dominant

arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and hereditary vascular retinopathy (HVR)[130-132]. Gene identification in the common forms of migraine has been extremely difficult, mainly because of the high prevalence, genetic heterogeneity and variable expression of the disorder. Furthermore, the consideration of patients with MA and / or MO attack types as being affected in families for linkage is an unresolved issue.

Mapping of migraine genes was initiated in Familial Hemiplegic Migraine; a rare autosomal dominant form of MA where patients additionally develop one-sided hemiparesis during attacks[16]. Two genes have been identified using this approach. The first gene (FHM1), CACNA1A, is located on chromosome 19p13 and encodes the $Ca_v2.1$ (formerly $\alpha 1A$) calcium channel subunit of P/Q-type calcium channels[22,58]. The second FHM gene (FHM2), ATP1A2, was identified on chromosome 1q23.2 and encodes the Na^+/K^+ ATPase $\alpha 2$ subunit[23,24]. Genome scans have also revealed several loci for the common types of migraine MA and MO. Loci identified in various single and multiple families were reported on chromosomes 1q31, 4q24, 6p12.2-p21.1, 11q24, 14q21.2-q22.3 and Xq24-q28 [133-139]. Recently, the Finnish MA locus on chromosome 4q24 has been replicated in MO families from Iceland[140]. Unfortunately, for none of the loci involved in the common types of migraine the causative gene has been identified yet.

Epilepsy

Epilepsy is characterized by recurrent unprovoked seizures with an abnormal electrical activity in the brain that leads to stereotype alterations in behavior[141]. The active epilepsy prevalence is 0.5% and is most often found in children and adolescents[142,143]. Epilepsy is a broad category of symptom complexes that arise from a large number of structural and functional brain disorders[144]. Epilepsy syndromes can be classified according to aetiology and seizure characteristics[18]. Different forms of seizures are: (1) *myoclonic seizures* during which a patient stares for a few seconds and sometimes blinks, (2) *atonic*

seizures during which a patient falls limply to the ground, (3) *tonic-clonic seizures* during which a patient becomes stiff and falls after which he has convulsions, and (4) *tonic seizures* which equal the tonic-clonic seizures except for the convulsions. Based on the aetiology, epilepsies can be put into the categories symptomatic, idiopathic and cryptogenic. Symptomatic are those epilepsies, which have a known underlying disorder, such as a stroke or tumors, and account for 20 to 40% of the epilepsy cases[141]. Idiopathic epilepsies are defined as epilepsies, which have no known underlying cause other than a hereditary predisposition. Cryptogenic are the epilepsies without any known associated risk factors and without presence of a familial predisposition. The epilepsy syndromes are characterized by combinations of clinical features like seizure types, age of onset and electroencephalogram (EEG) abnormalities.

Like for AD and migraine, familial studies and twin studies have shown that epilepsy is a disorder with genetic and environmental risk factors involved[145,146]. The estimated heritability of epilepsy ranges between 61 and 77% [12]. Of course, the contribution of genetic risk factors can vary with different epilepsy syndromes. Gene mapping studies have therefore focused on the idiopathic syndromes, which are frequently the rare monogenic variants of epilepsy syndromes. Positional cloning of the genes involved in these disorders has led to a multitude of mutations responsible for epilepsy[141]. Currently, nearly all known genes responsible for the epilepsy syndromes encode ion channels or functionally related structures. Examples are benign familial neonatal convulsions (BFNC) in which mutations have been found in the KCNQ2, KCNQ3 voltage gated potassium channels, or generalized epilepsy with febrile seizures (GEFS+) in which mutations have been described in the voltage gated sodium channels SCN1A, SCN1B and SCN2A[147-151]. However, for many other epilepsy syndromes the responsible genes have not been identified yet[152-154].

A part of the complexity of epilepsy syndromes is the overlap between various epilepsy syndromes that are described in literature. For example, in chapter eight a family is described, which fulfills the criteria of both autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) as well as familial partial epilepsy with variable foci (FPEVF)[155,156]. Furthermore, the variable expression of the syndrome(s) in patients, the reduced penetrance of the Mendelian forms of epilepsy and the substantial heterogeneity within the syndromes make the mapping of these genes a challenge.

Scope of the thesis

Complex neurological disorders are frequent in the population and have a substantial impact on health care, socio–economic level and quality of life. Finding genetic risk factors involved in these disorders may clarify the pathophysiology and biochemical pathways, and may boost knowledge about the disorder and possible treatment. The finding of genetic risk factors in complex neurological disorders is nonetheless often difficult. In this thesis, some methodological issues involved in studying complex neurological traits with association studies were addressed. In addition, family-based mapping techniques were applied to an assortment of pedigrees with complex neurological traits. In the ***first chapter*** a general introduction of the complex trait, its related problems with gene-mapping and the current methodology are discussed. The ***second chapter*** focuses on a problem that may be encountered in association studies: population stratification. A simple overview of methodology to test and, if necessary, circumvent population stratification is provided. Furthermore, the probability of finding false-positive association was studied in relation to population diversity and genetic drift. In the ***third chapter***, an approach is presented to evaluate false-positive gene-gene interactions found in association studies. This approach may greatly improve the study findings and detect statistical fluctuations in results. In ***chapter four*** the comorbidity and risk of migraine and Raynaud Phenomenon was studied with a locus involved in Hereditary Vascular Retinopathy. A TDT approach

was applied in a single family to study if the HVR haplotype would increase the susceptibility for both disorders. In ***chapter five*** segregation analyses were used to study how migraine attacks with - and without aura are inherited in Dutch migraine families. The effect of including patients with MA and MO in extended MO families was studied as well. In ***chapter six***, linkage analysis in seven large Dutch MO families was performed, which aimed at locating novel loci for migraine without aura. An interesting conclusion from this study is the confirmation of the Finnish locus on chromosome 4q24 known to be involved in MA. This study also showed the difficulties of linkage analysis in complex disease, as the heterogeneity of the disorder affected the linkage findings even under a homogeneous selection of families. In ***chapter seven*** heterogeneity of familial cortical tremor with epilepsy was shown with the exclusion of a Japanese locus on chromosome 8q23.3-q24.1. The mapping and replication of a locus for familial partial epilepsy with variable foci on chromosome 22q11-q12 in ***chapter eight*** shows that parametric linkage analysis in extended pedigrees can be a useful tool for mapping genes in more rare and less heterogeneous complex neurological disorders.

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