



Universiteit  
Leiden  
The Netherlands

## Genetic epidemiological approaches in complex neurological disorders

Hottenga, J.J.

### 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

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/4343>

**Note:** To cite this publication please use the final published version (if applicable).

# CHAPTER 1

## Introduction

JJ Hottenga



## **Complex neurological disorders**

The prevalence of various complex neurological disorders, like migraine and Alzheimer's disease, is high in the general population<sup>1-4</sup>. 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<sup>5-8</sup>. 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<sup>9</sup>. 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<sup>10</sup>.

## **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<sup>11-13</sup>. 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, nonsense, 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<sup>14</sup>. 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<sup>15-18</sup>. 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<sup>16</sup>. 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<sup>19,20</sup>. 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<sup>9,10,14</sup>. 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<sup>21</sup>. 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<sup>22-25</sup>.

### *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<sup>26,27</sup>. 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<sup>28-30</sup>. The sample sizes required for detecting interactions are substantial and may become prohibitive<sup>31</sup>. 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<sup>10,32,33</sup>.

## **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<sup>34</sup>.

### *Linkage studies in extended families*

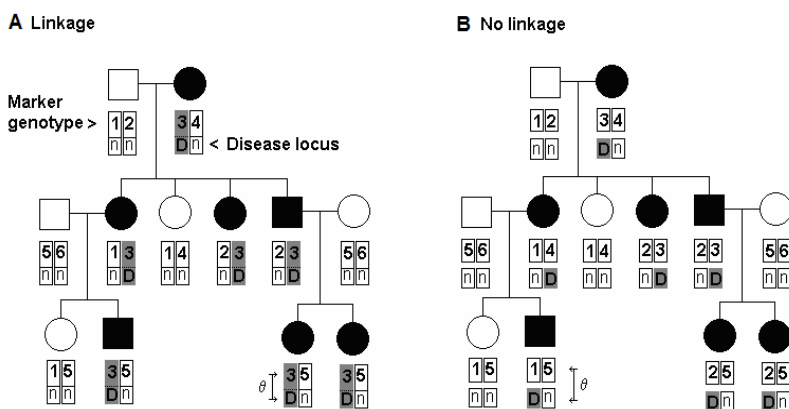
Hallmark of linkage analysis is a process called recombination. During meiosis, recombination occurs between homologous chromosomes in either parent leading to two new hybrid chromosomes (gametes) that are transmitted to the offspring<sup>35</sup>. In case one of the parents carries a risk gene, only 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 extent the disorder co-segregates with a tested marker allele (single point analysis) or multiple marker haplotype (multipoint analysis)<sup>36</sup>. 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<sup>33</sup>. 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  $\theta$ . 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<sup>37</sup>. In the model-based approach several parameters have to be specified in order to calculate the maximum likelihood for the linkage statistic<sup>36,37</sup>. 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<sup>38-40</sup>. 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<sup>41-43</sup>. Segregation analysis can be used to find the best fitting mode of inheritance and model parameters<sup>44-46</sup>. For parametric linkage analysis several programs, such as FASTLINK or MENDEL are available<sup>47-49</sup>. 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<sup>42,43</sup>. Non-parametric linkage for dichotomous traits or QTLs can be tested with programs like MENDEL, GENEHUNTER, MERLIN and SOLAR<sup>49-53</sup>.

Linkage analysis is sensitive to genetic heterogeneity<sup>54</sup>. 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<sup>16,55,56</sup>. 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<sup>55,57,58</sup>. 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<sup>36,59</sup>. Finally, locus homogeneity of studies may also be improved by selecting a sample from more homogenous isolated populations<sup>60,61</sup>.

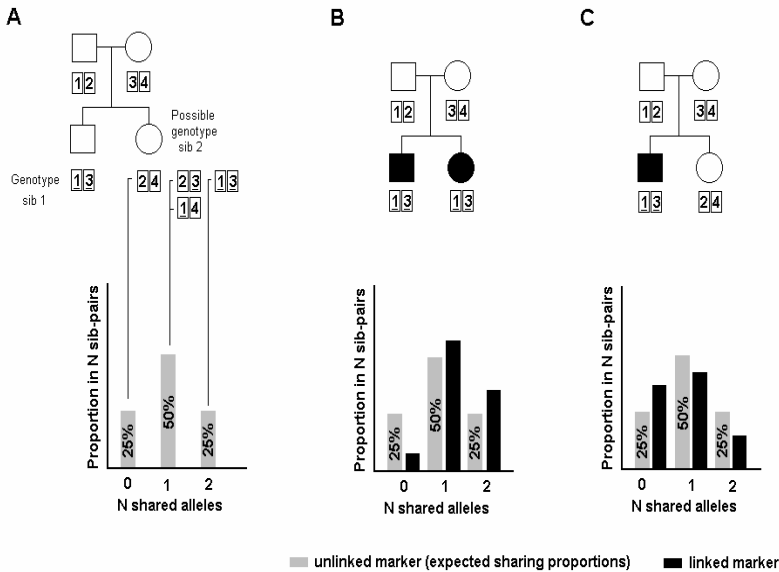
*Sib-pair studies*

In sib-pair studies the sharing of alleles between two siblings is studied in relation to the phenotype / disorder<sup>62</sup>. 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)<sup>63,64</sup>. Also QTLs can be studied where the trait variance between sibs is correlated with the number of shared alleles<sup>65-67</sup>. Affected sib-pair -, discordant sib-pair - and QTL analysis are implemented in various software packages like MAPMAKER SIBS, GENEHUNTER, MERLIN, MENDEL or SOLAR<sup>49-53,62</sup>. 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 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<sup>10</sup>. Unfortunately, the power to detect loci in complex disorders for this design is often low<sup>42,68</sup>. When a locus is detected, the shared region on the genome

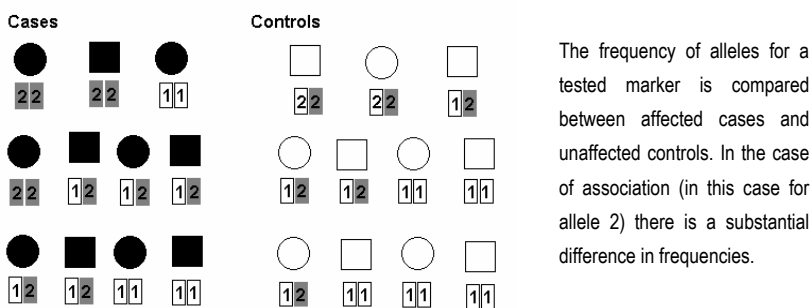
between two sibs is generally much larger compared to family or association studies hampering subsequent gene identification<sup>69</sup>.

### *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<sup>68</sup>. 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<sup>70,71</sup>. 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<sup>72-74</sup>.

**Figure 3**

The principle of an association study.



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<sup>75</sup>. 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<sup>76</sup>. These methods include the Pearson  $\chi^2$  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<sup>77</sup>. 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<sup>32,75</sup>. 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<sup>78,79</sup>. The expected shared genomic regions between cases are likely to be very small<sup>69</sup>. 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<sup>10,80,81</sup>. 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<sup>75,81-83</sup>. 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<sup>14,70,84,85</sup>. 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<sup>70</sup>.

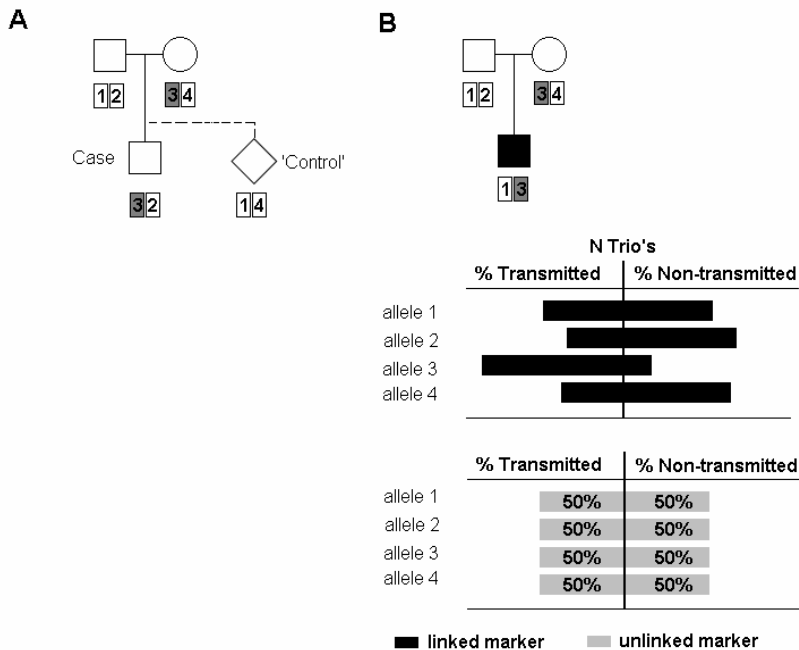
Several suggestions have been made to improve association study designs. These include testing for population stratification and other possible confounders, and to increase the significance level for reporting associations<sup>14,75,86,87</sup>. Also the study sample sizes, given the relative risks found for various associations, should be sufficiently high<sup>70,88</sup>. 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<sup>89,90</sup>. 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<sup>91</sup>. Another approach to the population stratification problem was the transmission disequilibrium test (TDT) (figure 4B)<sup>92</sup>.

**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  $\chi^2$  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<sup>91,93</sup>. 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<sup>94-97</sup>. Furthermore, the use of haplotypes / multiple markers with - or without known haplotype data of the parents has been proposed<sup>94,97</sup>. Other adjustments were made by various authors to incorporate QTLs or covariates like age and sex<sup>98-101</sup>. 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<sup>102-104</sup>. Family members were used both for reconstruction of parental genotypes/haplotypes, as well as to test the transmission over different family members<sup>94,102,105-107</sup>. 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<sup>93,108-111</sup>.

## **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<sup>3,4,112,113</sup>. Diagnosis of AD is made based on extensive clinical anamnesis following the NINCDS-ADRDA criteria<sup>17</sup>. The diagnosis can sometimes be ambiguous, as both vascular dementia and Parkinson's disease have a large clinical overlap with AD<sup>19,20</sup>. The pathology of AD shows extra cellular plaques mainly composed of amyloid  $\beta$  peptide and intracellular neurofibrillary tangles containing hyperphosphorylated protein<sup>114</sup>. 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<sup>13,115,116</sup>. 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%<sup>115</sup>. 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<sup>116</sup>.

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<sup>117,118</sup>. Subsequently, mutations in two other genes, Presenilin-1 and Presenilin-2

(PSEN1 and PSEN2), were identified on chromosomes 14q24 and 1q42, respectively<sup>119-121</sup>. 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<sup>26,70</sup>. 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<sup>122-124</sup>. Gene-gene interaction and gene-environment interaction, especially with APOE\*4 are frequently studied<sup>125-127</sup>. 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<sup>1,2</sup>. 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)<sup>16</sup>. 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<sup>128,129</sup>. The estimated heritability for the common types of migraine is 46%<sup>11</sup>. 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)<sup>130-132</sup>. 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<sup>16</sup>. Two genes have been identified using this approach. The first gene (FHM1), CACNA1A, is located on chromosome 19p13 and encodes the Ca<sub>v</sub>2.1 (formerly  $\alpha$ 1A) calcium channel subunit of P/Q-type calcium channels<sup>22,58</sup>. The second FHM gene (FHM2), ATP1A2, was identified on chromosome 1q23.2 and encodes the Na<sup>+</sup>/K<sup>+</sup> ATPase  $\alpha$ 2 subunit<sup>23,24</sup>. 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<sup>133-139</sup>. Recently, the Finnish MA locus on chromosome 4q24 has been replicated in MO families from Iceland<sup>140</sup>. 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<sup>141</sup>. The active epilepsy prevalence is 0.5% and is most often found in children and adolescents<sup>142,143</sup>. Epilepsy is a broad category of symptom complexes that arise from a large number of structural and functional brain disorders<sup>144</sup>. Epilepsy syndromes can be classified according to aetiology and seizure characteristics<sup>18</sup>. 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<sup>141</sup>. 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<sup>145,146</sup>. The estimated heritability of epilepsy ranges between 61 and 77%<sup>12</sup>. 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<sup>141</sup>. 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<sup>147-151</sup>. However, for many other epilepsy syndromes the responsible genes have not been identified yet<sup>152-154</sup>.

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)<sup>155,156</sup>. 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.

## References

1. Lipton RB, Stewart WF. Migraine headaches: epidemiology and co morbidity. *Clin Neurosci.* 1998;5:2-9.
2. Launer LJ, Terwindt GM, Ferrari MD. The prevalence and characteristics of migraine in a population-based cohort: the GEM study. *Neurology.* 1999;53:537-42.
3. Fitzpatrick AL, Kuller LH, Ives DG, et al. Incidence and prevalence of dementia in the Cardiovascular Health Study. *J Am Geriatr Soc.* 2004;52:195-204.
4. Bachman DL, Wolf PA, Linn R et al. Prevalence of dementia and probable senile dementia of the Alzheimer type in the Framingham Study. *Neurology.* 1992;42:115-9.
5. Breslau N, Rasmussen BK. The impact of migraine: Epidemiology, risk factors, and comorbidities. *Neurology.* 2001; 56 Suppl 1:S4-12.
6. Witthaus E, Ott A, Barendregt JJ et al. Burden of mortality and morbidity from dementia. *Alzheimer Dis Assoc Disord.* 1999;13:176-81.
7. Baker GA, Nashef L, van Hout BA. Current issues in the management of epilepsy: the impact of frequent seizures on cost of illness, quality of life, and mortality. *Epilepsia.* 1997;38 Suppl 1:S1-8.
8. Bishop M, Allen CA. The impact of epilepsy on quality of life: a qualitative analysis. *Epilepsy Behav.* 2003;4:226-33.

9. Khoury MJ, Beaty TH, Cohen BH. *Fundamentals of genetic epidemiology*. Oxford university press. Oxford. 1993. ISBN 0195052889.
10. Lander ES, Schork NJ. Genetic dissection of complex traits. *Science*. 1994;265:2037-48.
11. Mulder EJ, Van Baal C, Gaist D et al. Genetic and environmental influences on migraine: a twin study across six countries. *Twin Res*. 2003;6:422-31.
12. Kjeldsen MJ, Kyvik KO, Friis ML, Christensen K. Genetic and environmental factors in febrile seizures: a Danish population-based twin study. *Epilepsy Res*. 2002;51:167-77.
13. Pedersen NL, Gatz M, Berg S, Johansson B. How heritable is Alzheimer's disease late in life? Findings from Swedish twins. *Ann Neurol*. 2004;55:180-5.
14. Risch NJ. Searching for genetic determinants in the new millennium. *Nature*. 2000; 15;405:847-56.
15. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders DSM-IV-TR*. 4th edition. Arlington. 2000. ISBN 0890420254.
16. "International Headache Society". Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. *Cephalalgia* 1988;supplement 7 8:1-96.
17. McKhann G, Drachman D, Folstein M et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34:939-44.
18. Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia*. 1989;30:389-99.
19. Roman GC, Tatemichi TK, Erkinjuntti T et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology*. 1993;43:250-60.
20. Perl DP, Olanow CW, Calne D. Alzheimer's disease and Parkinson's disease: distinct entities or extremes of a spectrum of neurodegeneration? *Ann Neurol*. 1998;44 3 Suppl 1:S19-31.
21. Page GP, George V, Go RC et al. "Are we there yet?": Deciding when one has demonstrated specific genetic causation in complex diseases and quantitative traits. *Am J Hum Genet*. 2003;73:711-19.
22. Ophoff RA, Terwindt GM, Vergouwe MN et al. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca<sup>2+</sup> channel gene CACNL1A4. *Cell*. 1996;1;87:543-52.
23. De Fusco M, Marconi R, Silvestri L et al. Haploinsufficiency of ATP1A2 encoding the Na<sup>+</sup>/K<sup>+</sup> pump alpha2 subunit associated with familial hemiplegic migraine type 2. *Nat Genet*. 2003;33:192-6.
24. Ducros A, Joutel A, Vahedi K et al. Mapping of a second locus for familial hemiplegic migraine to 1q21-q23 and evidence of further heterogeneity. *Ann Neurol*. 1997;42:885-90.
25. Joutel A, Ducros A, Vahedi K et al. Genetic heterogeneity of familial hemiplegic migraine. *Am J Hum Genet*. 1994;55:1166-72.
26. Farrer LA, Cupples LA, Haines JL et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer's disease. A meta-analysis. APOE and Alzheimer's Disease Meta Analysis Consortium. *JAMA*. 1997;278:1349-56.
27. Kim KW, Jhoo JH, Lee KU et al. No association between presenilin 1 (PS1) intronic polymorphism and sporadic Alzheimer's disease in Koreans. *J Neural Transm*. 2000;107:1191-200.
28. Lucek P, Hanke J, Reich J et al. Multi-locus nonparametric linkage analysis of complex trait loci with neural networks. *Hum Hered*. 1998;48:275-84.



29. Beekman M, Heijmans BT, Martin NG et al. Two-locus linkage analysis applied to putative quantitative trait loci for lipoprotein(a) levels. *Twin Res.* 2003;6:322-4.
30. Liu X, Fallin MD, Kao WL. Genetic dissection methods: designs used for tests of gene-environment interaction. *Curr Opin Genet Dev.* 2004;14:241-45.
31. Gauderman WJ. Sample size requirements for association studies of gene-gene interaction. *Am J Epidemiol.* 2002;155:478-84.
32. Hirschhorn JN, Altshuler D. Once and again-issues surrounding replication in genetic association studies. *J Clin Endocrinol Metab.* 2002;87:4438-41.
33. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet.* 1995;11:241-7.
34. Blangero J. Localization and identification of human quantitative trait loci: king harvest has surely come. *Curr Opin Genet Dev.* 2004;14:233-40.
35. Strachan T, Read A. *Human Molecular Genetics.* 3rd edition. Garland Science/Taylor & Francis Group. New York. 2003. ISBN 0815341822.
36. Ott J, Terwilliger JD. *Handbook of human genetic linkage.* Baltimore, MD: Johns Hopkins University Press. 1994. ISBN 0801848032.
37. Ott J, Hoh J. Statistical approaches to gene mapping. *Am J Hum Genet.* 2000;67:289-94.
38. Weeks DE, Lehner T, Squires-Wheeler E, Kaufmann C, Ott J. Measuring the inflation of the lod score due to its maximization over model parameter values in human linkage analysis. *Genet Epidemiol.* 1990;7:237-43.
39. Clerget-Darpoux F, Babron MC, Bonaiti-Pellie C. Power and robustness of the linkage homogeneity test in genetic analysis of common disorders. *J Psychiatr Res.* 1987;21:625-30.
40. Risch N, Giuffra L. Model misspecification and multipoint linkage analysis. *Hum Hered.* 1992;42:77-92.
41. Greenberg DA, Abreu P, Hodge SE. The power to detect linkage in complex disease by means of simple LOD-score analyses. *Am J Hum Genet.* 1998;63:870-9.
42. Abreu PC, Greenberg DA, Hodge SE. Direct power comparisons between simple LOD scores and NPL scores for linkage analysis in complex diseases. *Am J Hum Genet.* 1999;65:847-57.
43. Hodge SE. Model-free vs. model-based linkage analysis: a false dichotomy? *Am J Med Genet.* 2001;105:62-4.
44. Morton NE, Rao DC, Lalouel JM. *Methods in genetic epidemiology.* S. Karger Publishing. New York. 1983. ISBN 3805536682.
45. Lalouel JM, Morton NE. Complex segregation analysis with pointers. *Hum Hered.* 1981;31:312-21.
46. Lalouel JM, Rao DC, Morton NE, Elston RC. A unified model for complex segregation analysis. *Am J Hum Genet.* 1983;35:816-26.
47. Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci U S A.* 1984;81:3443-6.
48. Lathrop GM, Lalouel JM. Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet.* 1984;36:460-5.
49. Lange K, Weeks D, Boehnke M. Programs for Pedigree Analysis: MENDEL, FISHER, and dGENE. *Genet Epidemiol.* 1988;5:471-2.
50. Li H, Schaid DJ. GENEHUNTER: application to analysis of bipolar pedigrees and some extensions. *Genet Epidemiol.* 1997;14:659-63.
51. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet.* 1996;58:1347-63.

52. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet.* 1998;62:1198-211.
53. Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet.* 2002;30:97-101.
54. Chiano MN, Yates JR. Linkage detection under heterogeneity and the mixture problem. *Ann Hum Genet.* 1995;59:83-95.
55. Bowcock AM, Anderson LA, Friedman LS et al. THRA1 and D17S183 flank an interval of < 4 cM for the breast-ovarian cancer gene (BRCA1) on chromosome 17q21. *Am J Hum Genet.* 1993;52:718-22.
56. Terwindt GM, Ophoff RA, Haan J et al. Familial hemiplegic migraine: a clinical comparison of families linked and unlinked to chromosome 19. DMGRG. *Cephalalgia.* 1996;16:153-5.
57. Brzustowicz LM, Hodgkinson KA, Chow EW et al. Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. *Science.* 2000;28:288:678-82.
58. Joutel A, Bousser MG, Biouesse V et al. A gene for familial hemiplegic migraine maps to chromosome 19. *Nat Genet.* 1993;5:40-5.
59. Bhat A, Heath SC, Ott J. Heterogeneity for multiple disease loci in linkage analysis. *Hum Hered* 1999;49:229-231.
60. Peltonen L, Palotie A, Lange K. Use of population isolates for mapping complex traits. *Nat Rev Genet.* 2000;1:182-90.
61. Vaessen N, Heutink P, Houwing-Duistermaat JJ et al. A genome-wide search for linkage-disequilibrium with type 1 diabetes in a recent genetically isolated population from the Netherlands. *Diabetes.* 2002;51:856-9.
62. Kruglyak L, Lander ES. Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am J Hum Genet.* 1995;57:439-54.
63. Risch N, Zhang H. Extreme discordant sib pairs for mapping quantitative trait loci in humans. *Science.* 1995;268:1584-9.
64. Zhao H, Zhang H, Rotter JJ. Cost-effective sib-pair designs in the mapping of quantitative-trait loci. *Am J Hum Genet.* 1997;60:1211-21.
65. Chen WM, Broman KW, Liang KY. Quantitative trait linkage analysis by generalized estimating equations: unification of variance components and Haseman-Elston regression. *Genet Epidemiol.* 2004;26:265-72.
66. Elston RC, Buxbaum S, Jacobs KB, Olson JM. Haseman and Elston revisited. *Genet Epidemiol.* 2000;19:1-17.
67. Putter H, Sandkuijl LA, van Houwelingen JC. Score test for detecting linkage to quantitative traits. *Genet Epidemiol.* 2002;22:345-55.
68. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science.* 1996;273:1516-7.
69. Houwen RH, Baharloo S, Blankenship K et al. Genome screening by searching for shared segments: mapping a gene for benign recurrent intrahepatic cholestasis. *Nat Genet.* 1994;8:380-6.
70. Lohmueller KE, Pearce CL, Pike M et al. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet.* 2003;33:177-82.
71. Botstein D, Risch N. Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. *Nat Gen.* 2003;33: Supplement 228-37.
72. Kennedy GC, Matsuzaki H, Dong S et al. Large-scale genotyping of complex DNA. *Nat Biotechnol.* 2003;21:1233-7.

73. International HapMap Consortium. Integrating ethics and science in the International HapMap Project. *Nat Rev Genet.* 2004;5:467-75.
74. John S, Shephard N, Liu G et al. Whole-genome scan, in a complex disease, using 11,245 single-nucleotide polymorphisms: comparison with microsatellites. *Am J Hum Genet.* 2004;75:54-64.
75. Little J, Bradley L, Bray MS et al. Reporting, appraising, and integrating data on genotype prevalence and gene-disease associations. *Am J Epidemiol.* 2002;156:300-10.
76. Rothman J, Greenland S. *Modern epidemiology.* 2nd edition. Lippincott Williams & Wilkins. Philadelphia. 1998. ISBN 0316757764.
77. Sasieni PD. From genotypes to genes: doubling the sample size. *Biometrics.* 1997;53:1253-61.
78. Long JR, Zhao LJ, Liu PY et al. Patterns of linkage disequilibrium and haplotype distribution in disease candidate genes. *BMC Genet.* 2004;5:11.
79. North BV, Curtis D, Martin ER, et al. Further Investigation of Linkage Disequilibrium SNPs and their Ability to Identify Associated Susceptibility Loci. *Ann Hum Genet.* 2004;68:240-8.
80. Knowler WC, Williams RC, Pettitt DJ, Steinberg AG. Gm3;5,13,14 and type 2 diabetes mellitus: an association in American Indians with genetic admixture. *Am J Hum Genet.* 1988;43:520-6.
81. Daly AK, Day CP. Candidate gene case-control association studies: advantages and potential pitfalls. *Br J Clin Pharmacol.* 2001;52:489-99.
82. Baron M. The search for complex disease genes: fault by linkage or fault by association? *Mol Psychiatry.* 2001;6:143-9.
83. Ioannidis JP. Genetic associations: false or true? *Trends Mol Med.* 2003;9:135-8.
84. Sachidanandam R, Weissman D, Schmidt SC et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature.* 2001;409:928-33.
85. Lander ES, Linton LM, Birren B et al. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. *Nature.* 2001;409:860-921.
86. Devlin B, Roeder K. Genomic control for association studies. *Biometrics.* 1999;55:997-1004.
87. Pritchard JK, Rosenberg NA. Use of unlinked genetic markers to detect population stratification in association studies. *Am J Hum Genet.* 1999;65:220-8.
88. Ioannidis JP, Trikalinos TA, Ntzani EE, Contopoulos-Ioannidis DG. Genetic associations in large versus small studies: an empirical assessment. *Lancet.* 2003;361:567-71.
89. Falk CT, Rubinstein P. Haplotype relative risks: an easy reliable way to construct a proper control sample for risk calculations. *Ann Hum Genet.* 1987;51:227-33.
90. Schaid DJ. Transmission disequilibrium, family controls, and great expectations. *Am J Hum Genet.* 1998;63:935-41.
91. Spielman RS, Ewens WJ. The TDT and other family-based tests for linkage disequilibrium and association. *Am J Hum Genet.* 1996;59:983-9.
92. Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet.* 1993;52:506-16.
93. Martin ER, Kaplan NL, Weir BS. Tests for linkage and association in nuclear families. *Am J Hum Genet.* 1997;61:439-48.
94. Lazzeroni LC, Lange K. A conditional inference framework for extending the transmission / disequilibrium test. *Hum Hered.* 1998;48:67-81.

95. Bickeboller H, Clerget-Darpoux F. Statistical properties of the allelic and genotypic transmission / disequilibrium test for multiallelic markers. *Genet Epidemiol.* 1995;12:865-70.
96. Kaplan NL, Martin ER, Weir BS. Power studies for the transmission/disequilibrium tests with multiple alleles. *Am J Hum Genet.* 1997;60:691-702.
97. Zhao H, Zhang S, Merikangas KR et al. Transmission / disequilibrium tests using multiple tightly linked markers. *Am J Hum Genet.* 2000;67:936-46.
98. Allison DB. Transmission-disequilibrium tests for quantitative traits. *Am J Hum Genet.* 1997;60:676-90.
99. Rabinowitz D. A transmission disequilibrium test for quantitative trait loci. *Hum Hered.* 1997;47:342-50.
100. Monks SA, Kaplan NL. Removing the sampling restrictions from family-based tests of association for a quantitative-trait locus. *Am J Hum Genet.* 2000;66:576-92.
101. Ghosh S, Reich T. The sib TDT adjusted for age of disease onset. *Ann Hum Genet.* 2004;68:249-56.
102. Curtis D. Use of siblings as controls in case-control association studies. *Ann Hum Genet.* 1997;61:319-33.
103. Spielman RS, Ewens WJ. A sibship test for linkage in the presence of association: the sib transmission / disequilibrium test. *Am J Hum Genet.* 1998;62:450-8.
104. Horvath S, Laird NM. A discordant-sibship test for disequilibrium and linkage: no need for parental data. *Am J Hum Genet.* 1998;63:1886-97.
105. Spielman RS, Ewens WJ. TDT clarification. *Am J Hum Genet.* 1999;64:668.
106. Knapp M. Using exact P values to compare the power between the reconstruction-combined transmission / disequilibrium test and the sib transmission/disequilibrium test. *Am J Hum Genet.* 1999;65:1208-10.
107. Knapp M. The transmission/disequilibrium test and parental-genotype reconstruction: the reconstruction-combined transmission/ disequilibrium test. *Am J Hum Genet.* 1999;64:861-70.
108. Martin ER, Monks SA, Warren LL, Kaplan NL. A test for linkage and association in general pedigrees: the pedigree disequilibrium test. *Am J Hum Genet.* 2000;67:146-54.
109. Laird NM, Horvath S, Xu X. Implementing a unified approach to family-based tests of association. *Genet Epidemiol.* 2000;19 Suppl 1:S36-42.
110. Rabinowitz D, Laird N. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. *Hum Hered.* 2000;50:211-23.
111. Schulze TG, McMahon FJ. Genetic association mapping at the crossroads: which test and why? Overview and practical guidelines. *Am J Med Genet.* 2002;114:1-11.
112. Rocca WA, Hofman A, Brayne C, et al. The prevalence of vascular dementia in Europe: facts and fragments from 1980-1990 studies. EURODEM-Prevalence Research Group. *Ann Neurol.* 1991;30:817-24.
113. van Duijn CM, Clayton D, Chandra V et al. Familial aggregation of Alzheimer's disease and related disorders: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. *Int J Epidemiol.* 1991;20 Suppl 2:S13-20.
114. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol (Berl).* 1991;82:239-59.
115. Pedersen NL, Posner SF, Gatz M. Multiple-Threshold Models for Genetic Influences on Age of Onset for Alzheimer Disease: Findings in Swedish Twins. *Am J Med Genet.* 2001;105:724-28

116. van Duijn CM, Farrer LA, Cupples LA, Hofman A. Genetic transmission of Alzheimer's disease among families in a Dutch population-based study. *J Med Genet.* 1993;30:640-6.
117. St George-Hyslop PH, Tanzi RE, Polinsky RJ et al. The genetic defect causing familial Alzheimer's disease maps on chromosome 21. *Science.* 1987;235:885-90.
118. Goate A, Chartier-Harlin MC, Mullan M et al. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature.* 1991;349:704-6.
119. Schellenberg GD, Bird TD, Wijsman EM et al. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science.* 1992;258:668-71.
120. Sherrington R, Rogaev EI, Liang Y et al. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature.* 1995;375:754-60.
121. Rogaev EI, Sherrington R, Rogaeva EA et al. Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature.* 1995;376:775-8.
122. Goddard KA, Olson JM, Payami H, Van Der Voet M, Kuivaniemi H, Tromp G. Evidence of linkage and association on chromosome 20 for late-onset Alzheimer disease. *Neurogenetics.* 2004;5:121-8.
123. Lendon C, Craddock N. Susceptibility gene(s) for Alzheimer's disease on chromosome 10. *Trends Neurosci.* 2001;24:557-9.
124. Pericak-Vance MA, Bass MP, Yamaoka LH, Gaskell PC, Scott WK et al. Complete genomic screen in late-onset familial Alzheimer disease. Evidence for a new locus on chromosome 12. *JAMA.* 1997 Oct 15;278:1237-41.
125. Mayeux R. Gene-environment interaction in late-onset Alzheimer disease: the role of apolipoprotein-epsilon4. *Alzheimer Dis Assoc Disord.* 1998;12 Suppl 3:S10-5.
126. Chandra V, Pandav R. Gene-environment interaction in Alzheimer's disease: a potential role for cholesterol. *Neuroepidemiology.* 1998;17:225-32.
127. Combarros O, Alvarez-Arcaya A, Oterino A et al. Polymorphisms in the presenilin 1 and presenilin 2 genes and risk for sporadic Alzheimer's disease. *J Neurol Sci.* 1999;171:88-91.
128. Gervil M, Ulrich V, Kaprio J et al. The relative role of genetic and environmental factors in migraine without aura. *Neurology.* 1999;53:995-9.
129. Ziegler DK, Hur YM, Bouchard TJ et al. Migraine in twins raised together and apart. *Headache.* 1998;38:417-22.
130. Verin M, Rolland Y, Landgraf F, et al. New phenotype of the cerebral autosomal dominant arteriopathy mapped to chromosome 19: migraine as the prominent clinical feature. *J Neurol Neurosurg Psychiatry.* 1995;59:579-85.
131. Dichgans M, Mayer M, Uttner I et al. The phenotypic spectrum of CADASIL: clinical findings in 102 cases. *Ann Neurol.* 1998; 44:731-9.
132. Terwindt GM, Haan J, Ophoff RA et al. Clinical and genetic analysis of a large Dutch family with autosomal dominant vascular retinopathy, migraine and Raynaud's phenomenon. *Brain.* 1998;121:303-16.
133. Lea RA, Shepherd AG, Curtain RP et al. A typical migraine susceptibility region localizes to chromosome 1q31. *Neurogenetics* 2002;4:1:17-22.
134. Wessman M, Kallela M, Kaunisto MA et al. A susceptibility locus for migraine with aura, on chromosome 4q24. *Am J Hum Genet* 2002;70:652-62.
135. Carlsson A, Forsgren L, Nylander PO et al. Identification of a susceptibility locus for migraine with and without aura on 6p12.2-p21.1. *Neurology* 2002;59:11:1804-7.
136. Cader ZM, Noble-Topham S, Dymant DA et al. Significant linkage to migraine with aura on chromosome 11q24. *Hum Mol Genet.* 2003;12:2511-7.

137. Soranga D, Vettori A, Carraro G et al. A locus for migraine without aura maps on chromosome 14q21.2-q22.3. *Am J Hum Genet.* 2003;72:161-7.
138. Nyholt DR, Dawkins JL, Brimage PJ et al. Evidence for an X-linked genetic component in familial typical migraine. *Hum Mol Genet* 1998;7:459-63.
139. Nyholt DR, Curtain RP, Griffiths LR. Familial typical migraine: significant linkage and localization of a gene to Xq24-28. *Hum Genet* 2000;107:18-23.
140. Bjornsson A, Gudmundsson G, Gudfinnsson E et al. Localization of a gene for migraine without aura to chromosome 4q21. *Am J Hum Genet.* 2003;73:986-93.
141. Tjink-Callenbach PMC. Clinical and genetic studies in hereditary epilepsy syndromes. Febodruk B.V. 2003. ISBN 9090172637.
142. Hauser WA. The prevalence and incidence of convulsive disorders in children. *Epilepsia.* 1994;35 Suppl 2:S1-6.
143. Wright J, Pickard N, Whitfield A, Hakin N. A population-based study of the prevalence, clinical characteristics and effect of ethnicity in epilepsy. *Seizure.* 2000;9:309-13.
144. McNamara JO. The neurobiological basis of epilepsy. *Trends Neurosci.* 1992;15:357-9.
145. Berkovic SF, Howell RA, Hay DA, Hopper JL. Epilepsies in twins: genetics of the major epilepsy syndromes. *Ann Neurol.* 1998;43:435-45.
146. Jain S, Padma MV, Puri A, Jyoti, Maheshwari MC. Occurrence of epilepsies in family members of Indian probands with different epileptic syndromes. *Epilepsia.* 1997;38:237-44.
147. Singh NA, Charlier C, Stauffer D et al. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat Genet.* 1998;18:25-9.
148. Hirose S, Zenri F, Akiyoshi H et al. A novel mutation of KCNQ3 (c.925T-->C) in a Japanese family with benign familial neonatal convulsions. *Ann Neurol.* 2000;47:822-6.
149. Wallace RH, Wang DW, Singh R et al. Febrile seizures and generalized epilepsy associated with a mutation in the Na<sup>+</sup>-channel beta1 subunit gene SCN1B. *Nat Genet.* 1998;19:366-70.
150. Escayg A, MacDonald BT, Meisler MH et al. Mutations of SCN1A, encoding a neuronal sodium channel, in two families with GEFS+2. *Nat Genet.* 2000;24:343-5.
151. Heron SE, Crossland KM, Andermann E et al. Sodium-channel defects in benign familial neonatal-infantile seizures. *Lancet.* 2002;360:851-2.
152. Cormier-Daire V, Dagoneau N, Nabbout R et al. A gene for pyridoxine-dependent epilepsy maps to chromosome 5q31. *Am J Hum Genet.* 2000;67:991-3.
153. Guipponi M, Rivier F, Vigevano F et al. Linkage mapping of benign familial infantile convulsions (BFIC) to chromosome 19q. *Hum Mol Genet.* 1997;6:473-7.
154. Zara F, Gennaro E, Stabile M et al. Mapping of a locus for a familial autosomal recessive idiopathic myoclonic epilepsy of infancy to chromosome 16p13. *Am J Hum Genet.* 2000;66:1552-7.
155. Scheffer IE, Bhatia KP, Lopes-Cendes I et al. Autosomal dominant nocturnal frontal lobe epilepsy. A distinctive clinical disorder. *Brain.* 1995;118:61-73.
156. Scheffer IE, Phillips HA, O'Brien CE et al. Familial partial epilepsy with variable foci: a new partial epilepsy syndrome with suggestion of linkage to chromosome 2. *Ann Neurol.* 1998;44:890-9.

