Genetics of Alzheimer's disease[1]

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Summary

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Alzheimer's disease is the most common cause of dementia in the elderly. Several potential factors have been described, which may determine the risk for the development of Alzheimer's disease. Among these, genetic factors play the most important role. From a genetic point of view, Alzheimer's disease may be subdivided into three forms according to the observed mode of inheritance: autosomal-dominant familial Alzheimer's disease, familial Alzheimer's disease without clear Mendelian inheritance (i.e. familial aggregation), and sporadic Alzheimer's disease without familial aggregation. This review article gives an overview of genetic research strategies in Alzheimer's disease, describes the known mutations resulting in an autosomal-dominant pattern of inheritance, and discusses some commonly accepted genetic susceptibility factors associated with the more common forms of Alzheimer's disease.

Keywords: genetics; polymorphism; association; linkage

Zusammenfassung

Die Demenz vom Alzheimer-Typ (DAT) ist die häufigste Ursache dementieller Syndrome in der älteren Allgemeinbevölkerung. Von allen potentiellen Faktoren, welche das Erkrankungsrisiko modifizieren können, spielen genetische Faktoren die wichtigste Rolle. Aus einem genetischen Standpunkt heraus kann die DAT je nach Vererbungsmodus in drei Formen unterteilt werden: autosomal-dominante familiäre DAT, familiäre DAT ohne klaren Mendelschen Vererbungsmodus (familiäre Aggregation) und sporadische DAT ohne familiäre Aggregation. Ziel dieses Übersichtsartikels ist es, einen Überblick über die genetischen Forschungsstrategien bei der DAT zu geben, die bekannten Mutationen, die zu einer autosomal-dominanten DAT führen, zu beschreiben und potentielle genetische Risikofaktoren, die mit der DAT assoziiert sind, zu diskutieren.

Schlüsselwörter: Genetik; Polymorphismus; Assoziation; Kopplung

Introduction

Alzheimer's disease is a neurodegenerative disorder, which preferentially affects individuals over 60 years of age with steadily increasing risk in older age groups. The prevalence of Alzheimer's disease in the general population increases from 1% in persons younger than 65 years to approximately 40% in nonagenarians [1].

Clinically, Alzheimer's disease is characterised by progressive cognitive deficits such as impairment of memory and orientation. With disease progression, non-cognitive symptoms such as delusions, agitation, changes in personality, and mood disturbances may also occur.

Neuropathologically, Alzheimer's disease is characterised by the presence of two histologic hallmarks: neuritic plaques and neurofibrillary tangles. Aggregates of high-grade fibrillar forms of β-amyloid peptide (Aβ) build the core of neuritic plaques. Enhanced Aβ production seems to be a central pathophysiological step in the Alzheimer's disease-related neurodegenerative cascade. The production of Aβ, which is derived from the amyloid precursor protein (APP), is under the control of the proteolytic activity of the alpha-, beta-, and gamma-secretases. While the

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alpha-secretase cleavage site precludes the formation of Aβ, beta- and gamma-secretases generate amyloidogenic APP components.

Principles of Alzheimer's disease genetics

The aetiology of Alzheimer's disease is multifactorial. Several factors exist, which determine the risk for the development of Alzheimer's disease and modify the age-at-onset and the course of the disease. These factors may be:

1 genetic (e.g. predisposing risk alleles),
2 sociodemographic (e.g. school education),
3 life style (e.g. nutritional aspects),
4 environment (e.g. head trauma),
5 clinical (e.g. comorbidity and medical history),
6 medication (e.g. influence of non-steroidal anti-inflammatory drugs on Alzheimer's disease development).

Taking into account the amount of potential risk factors and their possible interactions, the level of disease complexity could be very high. However, the contribution of genetic factors seems to be considerable: 74% of the risk for late-onset Alzheimer's disease (i.e. onset after the 65th year) are estimated to be genetic [2].

Modes of inheritance

From a genetic point of view, Alzheimer's disease may be subdivided into three forms according to the observed mode of inheritance within families:

1 autosomal-dominant familial Alzheimer's disease,
2 familial Alzheimer's disease without clear Mendelian inheritance (familial aggregation),
3 sporadic Alzheimer's disease without familial aggregation.

Only a minority of all Alzheimer's disease cases may be fully explained by the presence of genetic factors (autosomal-dominant Alzheimer's disease). These cases are caused by mutations in the genes encoding APP, *presenilin 1 (PSEN1),* and *presenilin 2 (PSEN2).* Several studies demonstrated the existence of familial aggregation, in that relatives of Alzheimer's disease patients show increased risk for developing dementia compared with relatives of healthy control subjects [3–11]. The familial aggregation of Alzheimer's disease may be due to shared genetic or, at least theoretically, environmental risk factors within families. Most cases are, however, supposed to be sporadic, which is defined by the absence of evidence for familial aggregation.

Research strategies

There are generally two strategies for examining genetic risk factors of complex and common diseases: linkage studies and association studies.

In *linkage studies,* genetic markers which may cover the entire genome at a given resolution are examined in families with multiple affected members. If a certain marker is close to a disease-causing mutation, it is unlikely that during recombination these two genetic loci will be separated. As a result, both loci will be inherited together (linkage disequilibrium) and the marker will be associated with the disease trait within families. Since the chromosomal localisation of the marker is known, positional cloning will identify the hitherto unknown disease-causing mutation. The strategy of linkage analysis and subsequent positional cloning has been very successful in monogenic disorders and in some rare and severe variants of complex diseases. However, the use of linkage mapping in common and complex diseases may be problematic: the polygenic aetiology of these diseases reduces the possibility that a marker in linkage disequilibrium with a putative susceptibility locus will produce a sufficient signal for statistical detection. Even if a signal is detected, subsequent positional cloning is impeded due to the considerable inaccuracy with respect to the correct localisation of the target gene (the target gene may be located within a region of 40cM around the marker) [12]. Furthermore, practical problems, which are inherent to Alzheimer's disease research (late onset of disease, lack of sufficiently large informative families, uncertain diagnoses), may additionally reduce the feasibility of linkage studies.

Association studies follow a different strategy: based on theoretical considerations and experimental findings, a gene (or a number of genes) involved in Alzheimer's disease pathogenesis is chosen. Variations of this gene, which ideally alter gene function, are expected to be associated with the risk for the development of disease either as protective or as risk factors. This hypothesis can be tested by assessment of the frequency of the genetic variation in a sample of Alzheimer's disease patients and control subjects. There are many advantages inherent to association studies:

1 The selection of the candidate gene is plausible and based on empirical background: the focus may be upon biologically defined candidate genes, genes suggested by differential display experiments, or positional candidates from prior linkage studies.
2 In common diseases, the signal derived from association studies is expected to be greater

than that derived from linkage studies. For example, numerous association studies identified the apolipoprotein E (apoE) ε4-allele (apoE4) to be a risk factor for Alzheimer's disease with an odds ratio of 3 to 4. Linkage studies failed to replicate this finding with appropriate statistical significance or accuracy of the identified chromosomal locus.

3 The genotyping and statistical methods used in association studies are easy to perform, thus enabling independent replication experiments.

However, there are two major issues, which must be considered when interpreting the results of association studies:

1 The validity of an association study depends critically upon a proper selection of patients and control subjects. While matching for age, sex, and educational level is an easy-to-achieve prerequisite, controlling for ethnicity (i.e. similar genetic background) may become a problem, especially in population-based association studies. Population admixture is difficult to control for and may lead to erroneous results.

2 The number of possible candidate genes, which can be examined in a case-control sample, is very high, thus many false-positive results may be generated. Indeed, there is a considerable number of studies reporting a significant association of a genetic variant with Alzheimer's disease, whereas the number of at least partially replicated findings is limited.

The development of family-based association tests aims at dealing with the problem of population stratification. The recently developed Sibship Disequilibrium Test (SDT) [13] does not require parental data and is therefore especially useful in genetic studies on Alzheimer's disease. It uses all the siblings in a sibship, remains valid even in the presence of misclassification of the affection status, and does not detect spurious associations due to population stratification.

Genetics of sporadic Alzheimer's disease

Sporadic Alzheimer's disease accounts for the majority of all Alzheimer's disease cases. Genetic factors seem to influence the risk for the development of sporadic Alzheimer's disease and case-control genetic association studies are broadly used for their assessment. Usually, the selection of candidate genes examined in association studies is hypothesis driven and based upon pathophysiological criteria. In the case of sporadic Alzheimer's disease, most candidate genes are involved in amyloid metabolism (alpha-2-macroglobulin [A2M]; cystatin C [CST3]; low-density lipoprotein-related protein 1 [LRP1]; apolipoprotein E [APOE]; cathepsin D [CTSD]) or mediate the Alzheimer's disease-related immune reaction (interleukin 1 alpha [IL1A]; interleukin 6 [IL6]). APOE is the only hitherto well-established risk factor for sporadic Alzheimer's disease. Research findings on the other genes remain controversial.

Apolipoprotein E: APOE

APOE plays a central role in the regulation of the cholesterol and triglyceride metabolism [14]. Three alleles of the gene encoding APOE have been described on the basis of two singe nucleotide polymorphisms, resulting in two amino acid changes at positions 112 and 158. The APOE ε2-allele is characterised by cysteine at positions 112 and 158, the APOE ε3-allele by cysteine at position 112 and arginine at position 158, and the APOE ε4-allele by arginine at both positions.

A significant association of the APOE ε4-allele with Alzheimer's disease was initially demonstrated in 1993 [15, 16]. This finding is hitherto the best-established genetic association with Alzheimer's disease and was replicated in several subsequent studies. In Caucasian populations, APOE ε4 heterozygous individuals have a threefold increased risk and homozygous persons an approximately eightfold increased risk for developing Alzheimer's disease by age 75 compared to APOE ε3 heterozygous individuals. The magnitude of the effect of the APOE ε4-allele as a risk factor for Alzheimer's disease is age and ethnicity dependent. Although the APOE ε4-effect is evident at all ages between 40 and 90, it becomes weaker after the age of 70. The highest odds ratios (ORs) are detected in the Japanese population (OR = 5.6 for APOE ε4-allele heterozygous, OR = 33.1 for APOE ε4-allele homozygous) [17]. The APOE ε4-effect is attenuated in Hispanic populations (OR = 2.5 for APOE ε4-allele homozygous), and, interestingly, in some African populations no association between APOE ε4 and Alzheimer's disease can be observed [18, 19]. These results together with the observation that the frequency of the APOE ε4-allele in ethnical groups like Pygmies, aborigines of Malaysia and Australia, Papuans, some Native Americans, and Sami is significantly higher than in populations with long-established agricultural economy [20] indicate that the association between Alzheimer's disease and the APOE ε4-allele can be explained in part by the interaction of this allele with contemporary environmental conditions.

Table 1 *APP* gene mutations in autosomal-dominant Alzheimer's disease.

exon	mutation	authors	citation
16	K670N	Mullan et al., 1992	[54]
16	M671L	Mullan et al., 1992	[54]
17	A692G	Hendriks et al., 1992	[55]
17	A693Q	Levy et al., 1990	[36]
17	I716V	Eckman et al., 1997	[56]
17	V717I	Goate et al., 1991	[57]
17	V717G	Chartier-Harlin et al., 1991	[58]
17	V717L	Murrell et al., 2000	[59]

Due to the high odds ratios, at least in Caucasian populations, the possibility of using the APOE genotype as a diagnostic tool has been considered [21, 22]. However, as stated by the American College of Medical Genetics/American Society of Human Genetics Working Group on ApoE and Alzheimer disease [23], the APOE genotype is not suitable for genetic testing because of its low sensitivity (65%) and specificity (65%) for Alzheimer's disease [24].

Alpha-2-macroglobulin: A2M

Alpha-2-macroglobulin is a mediator of Aβ clearance and degradation. In a family-based association study, the gene encoding alpha-2-macroglobulin, in particular a pentanucleotide deletion, was introduced as a susceptibility factor for Alzheimer's disease [25]. Although this association was partially replicated [26], no association was demonstrated in subsequent studies [27–29]. A2M is located in a chromosomal region (chromosome 12), which has been suggested as a region-of-interest in previous genome scans. The failures to replicate the initial association suggest that the pentanucleotide deletion of A2M may be in linkage disequilibrium with another polymorphism of A2M or a closely related gene on chromosome 12.

Additional risk genes for LOAD

Similar findings have been obtained for several additional genes, which are involved in the Alzheimer's disease-related pathophysiologic cascade, such as the low-density lipoprotein receptor-related protein (LRP1), cystatin C (CST3), cathepsin D (CTSD), bleomycin hydrolase (BLMH), interleukin 6 (IL6), and interleukin 1 (IL1). Polymorphisms of these genes have been suggested as potential susceptibility factors for Alzheimer's disease, which may also modify the onset of the disease [30].

Currently, a locus on chromosome 10 is discussed as an important risk factor in LOAD [31–33]. It is generally accepted that several genes modify the risk for the development of Alzheimer's disease. The recent advances in high-throughput genotyping methods together with the ongoing discovery of single nucleotide polymorphisms throughout the human genome will help in identifying the majority of these genes in the near future.

Genetics of autosomal-dominant Alzheimer's disease

It has been known for many years that in a small number of families worldwide, Alzheimer's disease is inherited as a fully penetrant, autosomal-dominant disease apparently resulting from a single gene defect (i.e. gene mutations by themselves sufficient to cause Alzheimer's disease). Irrespective of their low frequency, these monogenic Alzheimer's disease families have been of utmost importance for the identification of causative Alzheimer's disease genes by using the methods of linkage analysis with subsequent positional cloning. This strategy has led to the identification of 3 hitherto known Alzheimer's disease genes.

Amyloid precursor protein (APP)

In 1987, St George-Hyslop et al. [34], located a genetic defect causing autosomal-dominant Alzheimer's disease on the long arm of chromosome 21. The *APP* gene, which codes for the amyloid precursor protein, was found to map in this region [35]. Interestingly, a mutation in exon 16 of *APP* was found to cause hereditary cerebral haemorrhage with amyloidosis of the Dutch type (HCHWA-D) [36]. HCHWA-D is associated with Aβ deposition in cerebral blood vessels with the consequence of recurrent cerebral haemorrhages. Moreover, amyloid plaques similar to those found in Alzheimer's disease patients were described in the brain of patients with HCHWA-D. These observations strongly supported the notion that the likelihood of *APP* mutations also causing Alzheimer's disease would be very high. In 1991, Goate et al. described the first missense mutation in exon 17 of *APP* cosegregating with familial Alzheimer's disease. Subsequent studies identified

additional *APP* mutations in families with presenile Alzheimer's disease [37]. Interestingly, all these mutations are located in exons 16 and 17 of the *APP*, which encode the Aβ region of APP (table 1). By altering the proteolytic cleavage of the Aβ region, these mutations result in overproduction of the amyloidogenic, 42 amino acids-long Aβ (Aβ42). Despite extensive searching, no *APP* mutations away from the sites of proteolytic cleavage of the Aβ region have been discovered so far. Although *APP* mutations are sufficient to cause Alzheimer's disease, their effect may be additionally modified by gene–gene interactions: in the majority of families bearing *APP* mutations, the APOE ε4-allele results in an earlier age at onset.

The identification of *APP* mutations was instrumental for the understanding of the metabolic

Table 2 *PSEN1* gene mutations in autosomal-dominant Alzheimer's disease.

exon	mutation	authors	citation
4	A79V	Cruts and Van Broeckhoven, 1998	[60]
4	V82L	Campion et al., 1995	[61]
4	V96F	Kamino et al., 1996	[62]
5	F105L	Finckh et al., 2000	[63]
5	L113P	Raux et al., 2000	[64]
5	Y115H	Campion et al., 1995	[61]
5	Y115C	Cruts and Van Broeckhoven, 1998	[60]
5	T116N	Romero et al., 1999	[65]
5	P117L	Wisniewski et al., 1998	[66]
5	E120K	Hutton et al., 1996	[67]
5	E120D	Reznik-Wolf et al., 1996	[48]
5	K123E	Yasuda et al., 1999	[68]
5	M139V	Boteva et al., 1996	[69]
5	M139T	Campion et al., 1995	[61]
5	M139I	Boteva et al., 1996	[69]
5	I143F	Rossor et al., 1996	[70]
5	I143T	Cruts et al., 1995	[71]
5	I143F	Palmer et al., 1999	[72]
5	M146L	Sorbi et al., 1995	[73]
5	M146L	Sherrington et al., 1995	[40]
5	M146V	Alzheimer's Disease Collaborative Group, 1995	[74]
5	M146I	Jorgensen et al., 1996	[75]
5	L153V	Raux et al., 2000	[76]
6	H163Y	Alzheimer's Disease Collaborative Group, 1995	[74]
6	H163R	Sherrington et al., 1995	[40]
6	L166R	Ezquerra et al., 2000	[77]
6	S169L	Taddei et al., 1998	[78]
6	S169P	Ezquerra et al., 1999	[44]
6	L171P	Ramirez-Duenas et al., 1998	[79]
7	G209V	Younkin et al., 1996	[80]
7	G209R	Sugiyama et al., 1999	[81]
7	I213T	Kamino et al., 1996	[62]
7	L219P	Smith et al., 1999	[82]
7	A231T	Campion et al., 1995	[61]
7	A231V	Cruts and Van Broeckhoven, 1998	[60]
7	M233T	Kwok et al., 1997	[83]
7	M233L	Aldudo et al., 1999	[84]
7	L235P	Campion et al., 1995	[61]
7	A246E	Sherrington et al., 1995	[40]
7	L250S	Hutton et al., 1996	[67]
8	A260V	Rogaev et al., 1995	[53]
8	L262P	Forsell et al., 1997	[85]
8	C263R	Wasco et al., 1995	[86]
8	P264L	Campion et al., 1995	[61]
8	P267S	Alzheimer's Disease Collaborative Group, 1995	[74]
8	R269G	Perez-Tur et al., 1996	[87]
8	R269H	Gomez-Isla et al., 1997	[88]
8	R278T	Kwok et al., 1997	[83]
8	E280A	Alzheimer's Disease Collaborative Group, 1995	[74]
8	E280G	Alzheimer's Disease Collaborative Group, 1995	[74]
8	L282R	Aldudo et al., 1998	[89]
8	A285V	Rogaev et al., 1995	[53]
8	L286V	Sherrington et al., 1995	[40]
	Δ9	Perez-Tur et al., 1995	[90]
9	E318G	Sandbrink et al., 1996	[91]
11	G378E	Besancon et al., 1998	[92]
11	G384A	Cruts et al., 1995	[71]
11	L392V	Rogaev et al., 1995	[53]
11	L392P	Tedde et al., 2000	[93]
11	A409T	Aldudo et al., 1999	[84]
11	C410Y	Campion et al., 1995	[61]
12	L424R	Kowalska et al., 1999	[94]
12	A426P	Poorkaj et al., 1998	[95]
12	434	Devi et al., 2000	[96]
12	P436S	Palmer et al., 1999	[72]
12	P436Q	Taddei et al., 1998	[78]

Table 3 *PS2* gene mutations in autosomal-dominant Alzheimer's disease.

exon	mutation	authors	citation
5	N141I	Levy-Lahad et al., 1995	[51]
5	V148I	Lao et al., 1998	[97]
7	M239I	Finckh et al., 2000	[63]
7	M239V	Rogaev et al., 1995	[53]

cascades leading to enhanced Aβ production and gave rise to the amyloid hypothesis of Alzheimer's disease [38]. However, only a small percentage of autosomal-dominant Alzheimer's disease is caused by *APP* mutations. Linkage analysis excluding families with *APP* mutations led to the identification of a novel gene family, the presenilins.

Presenilin 1 (PSEN1)

A genetic locus involved in early-onset autosomal-dominant Alzheimer's disease was identified on the long arm of chromosome 14 by Schellenberg et al. in 1992 [39]. Positional cloning and examination of various transcripts of this chromosomal region led to the discovery of the *presenilin 1* (*PSEN1*) gene on 14q24.3, which contained five different missense mutations cosegregating with early-onset autosomal-dominant Alzheimer's disease [40]. Since then, several *PSEN1* mutations in over 80 families have been identified, resulting in an aggressive, early form of the disorder between ages 35 and 65. Thus, *PSEN1* mutations account for the majority of autosomal-dominant Alzheimer's disease cases (table 2). All but two presenilin mutations are missense mutations scattered throughout the molecule. However, they tend to cluster within and in the vicinity of the transmembrane domains, which are important for the protein activity of the presenilins.

The *APOE* gene does not seem to interact with *PSEN1* and does not further influence the onset age in *PSEN1* families [41]. However, other factors – probably of genetic origin – seem to interfere with *PSEN1*, since a considerable phenotypic variability (e.g. variable age of onset and variable clinical presentation) may be observed even within families carrying a specific mutation [42–48]. Moreover, some *PSEN1* mutations show incomplete penetrance, since not all mutation carriers will ultimately develop the disease.

After the identification of *PSEN1* as a causative Alzheimer's disease gene, the mechanism by which mutations of this gene caused the Alzheimer's disease phenotype was an open matter and not necessarily linked directly to Aβ production. Studies on fibroblast cell cultures of *PSEN1* mutation carriers revealed a marked elevation of Aβ42 levels, suggesting that presenilin function is important for the regulation of APP processing [49]. Modelling of *PSEN1* mutations in cultured cells and in transgenic mice supported the notion that presenilin cleavage has a direct effect on APP procession and Aβ production. It is currently well-accepted that presenilins influence γ-secretase activity or may in fact be γ-secretase.

Presenilin 2 (PSEN2)

In 1988, Bird et al. described a group of families with autosomal-dominant early-onset Alzheimer's disease descending from a single German family that first immigrated to Russia and later to the United States ("founder effect") [50]. Neither *APP* nor *PSEN1* mutations were detected in these Volga-German kindreds. The search for proteins homologous to *presenilin 1* led to the cloning and characterisation of the *PSEN2* gene, which is located on chromosome 1q31–q42 and codes for a protein highly homologous to *presenilin 1* [51, 52]. Four missense *PSEN2* mutations have been described so far, one in Volga-German families and three others in two Italian families and one Dutch family (table 3) [53, 63]. In analogy to *PSEN1*, the *APOE* gene does not seem to interact with *PSEN2* and does not further influence the onset age in *PSEN2* families. Similarly, incomplete penetrance and variable age of onset and clinical presentation are also characteristics of *PSEN2* mutations.

Conclusion

Alzheimer's disease is a genetically heterogeneous disorder, in that several genes contribute to the disease risk. The identification of families with an autosomal-dominant mode of inheritance has been instrumental in the search for genetic defects and has led to the discovery of disease-causing mutations in the *APP*, *PSEN1* and *PSEN2* genes by linkage analysis and positional cloning. Most Alzheimer's disease cases, however, do not follow a clear Mendelian mode of inheritance and are considered polygenic diseases. The ε4-allele of the APOE genotype is the best-established genetic risk factor in these cases. However, it is obvious that several other genes, each exerting a minor effect, contribute to the overall genetically determined risk for the development of the disease. The recent advances in the characterisation of the human genome, the identification of a large amount of

polymorphic sites throughout human DNA and the development of high-throughput genotyping methods and elaborated statistical analyses will ultimately allow deciphering these genetic susceptibility factors. It is possible that in the near future, genetic research will contribute to the estimation of individual disease risks and to the optimisation of therapeutic strategies.

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