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Genetics, transcriptomics and proteomics of Alzheimer's disease

Andreas Papassotiropoulos, M.D.[1,3], **Michael Fountoulakis, Ph.D.**[2], **Travis Dunckley, Ph.D.**[3,5], **Dietrich A. Stephan, Ph.D.**[3,5], and **Eric M. Reiman, M.D.**[3,4,5]

[1]*Division of Psychiatry Research, University of Zurich, Switzerland* [2]*Foundation for Biomedical research of the Academy of Athens, Greece* [3]*Neurogenomics Division, The Translational Genomics Research Institute, Phoenix AZ, USA* [4]*The Banner Alzheimer's Disease Institute and the Department of Psychiatry, University of Arizona, AZ, USA* [5]*Arizona Alzheimer's Disease Consortium, AZ, USA*

Abstract

Objective—To provide an updated overview of the methods used in genetic, transcriptomic and proteomic studies in Alzheimer's disease and to demonstrate the importance of those methods for the improvement of the current diagnostic and therapeutic possibilities.

Data sources—Medline-based search of 234 peer-reviewed articles published between 1975 and 2006.

Data synthesis—Alzheimer's disease is a genetically heterogeneous disorder. Rare mutations in the APP, presenilin 1, and presenilin 2 genes have shown the importance of the amyloid metabolism for its development. In addition, converging evidence from population-based genetic studies, gene expression studies and protein profile studies in the brain and in the cerebrospinal fluid suggest the existence of several pathogenetic pathways such as amyloid precursor protein processing, β-amyloid degradation, tau phosphorylation, proteolysis, protein misfolding, neuroinflammation, oxidative stress and lipid metabolism.

Conclusions—The development of high-throughput genotyping methods and of elaborated statistical analyses will contribute to the identification of genetic risk profiles related to the development and course of this devastating disease. The integration of knowledge derived from genetic, transcriptomic and proteomic studies will greatly advance our understanding of the causes of Alzheimer's disease, it will improve our capability of establishing an early diagnosis, it will help defining disease subgroups and it will ultimately help to pave the road towards improved and tailored treatments.

I. Genetics of Alzheimer's disease

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

Clinically, AD is characterized by progressive impairments in memory and other cognitive domains. With disease progression, non-cognitive symptoms such as delusions, agitation, changes in personality, and mood disturbances may also occur. Neuropathologically, AD is characterized by the presence of two histological hallmarks, neuritic plaques and neurofibrillary tangles. Aggregates of fibrillar β-amyloid peptide (Aβ) form the core of the neuritic plaques. The accumulation of Aβ (particularly the aggregated form of the protein

Corresponding author: Andreas Papassotiropoulos, M.D., Division of Psychiatry Research, University of Zürich, Lenggstrasse 31, PO Box 1931, CH-8032 Zürich, Switzerland, Phone: +41-44-384-2644, FAX: +41-44-384-2686, e-mail: papas@bli.unizh.ch.

containing 42 amino acids) has been strongly suggested to play a central pathophysiological role in the AD-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 alpha-secretase cleavage site precludes the formation of Aβ, beta- and gamma-secretases generate amyloidogenic APP components.

Ia. Principles of AD genetics

AD is a multifactorial and genetically complex disorder. Several factors influence the risk for the development of AD and modify the age-at-onset and the course of the disease. These factors may be:

1. genetic (e.g. causative mutations, predisposing risk alleles, protective alleles)
2. sociodemographic (e.g. level of education, intelligence)
3. life style (e.g. aspects of nutrition, aerobic fitness, and mental exercise)
4. environment (e.g. head trauma)
5. clinical (e.g. comorbid medical conditions)
6. medications (e.g. non-steroidal anti-inflammatory drugs, statins)

Of these factors, genetic influences seem to be of major importance: twin studies suggest that about 74% of the risk for late-onset AD (i.e. onset after the 65th year) is genetic.[2]

Modes of inheritance—From a genetic point of view, AD may be subdivided into three forms according to the observed mode of inheritance within families:

1. autosomal-dominant familial AD
2. familial AD without clear mendelian inheritance (familial aggregation)
3. sporadic AD without familial aggregation

Only a minority of all AD cases may be fully explained by the presence of genetic factors (autosomal dominant AD). Most of these cases are caused by mutations in the genes encoding the amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*). Several studies demonstrated the existence of familial aggregation, such that relatives of AD patients show increased risk for developing dementia compared with relatives of healthy control subjects.[3–12] The familial aggregation of AD may be due to shared genetic or, at least theoretically, environmental risk factors within families. Most cases are, however, 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 are examined in families with multiple affected members. If a certain marker with known chromosomal localization is linked to the disease due to linkage disequilibrium with the disease-causing mutation, positional cloning and fine-mapping is performed to identify the disease-related gene. 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 complex traits 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 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 localization of the target gene (the target gene may be located within a region of 40cM around the marker).[13] Furthermore,

practical problems, which are inherent to AD research (late onset of disease, lack of sufficient 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, one or more genes involved in AD pathogenesis are chosen (candidate-genes). Polymorphisms of these genes, which ideally alter gene function, are expected to be associated with the risk for the development of disease. This hypothesis can be tested by assessment of the frequency of the genetic variation in a sample of AD patients and control subjects. Association mapping is an additional and hypothesis-free method of genetic association. It is used to detect chromosomal regions containing disease-susceptibility loci through the identification of marker loci at which some alleles are more frequent among cases than among controls. There are many advantages inherent to the genetic association approach to the study of AD:

1. The selection of candidate genes is plausible and based on empirical data about the molecular events contributing to AD histopathology: 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*) e4 allele (*APOE*4) to be a risk factor for AD with an odds ratio of 3 to 4, and persons with two copies of this allele have an even higher odds ratio.[14] Several linkage studies failed to detect this signal.
3. The genotyping and statistical methods used in association studies are easy to perform, thus enabling independent replication experiments.
4. As noted below, the development of high-density genotyping platforms with the ability to genotype >500,000 single nucleotide polymorphisms (SNPs) may, for the first time, provide sufficiently dense markers to permit an unbiased hypothesis-free association study of the entire human genome. It has been proposed that one needs at least 300,000 evenly distributed markers to conduct a whole genome study (assuming an adequate number of well characterized cases and controls are included).

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, gender, 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. The development of such family-based association tests as the sibship disequilibrium test (TDT)[15] and others aims at dealing with the problem of population stratification. In addition, methods such as the Structured Association analysis[16] and Genomic Control[17] may be used in case-control association studies to estimate the level of genetic heterogeneity and thereby to control for this possible bias.
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, a considerable number of studies reported significant associations of genetic variants with AD, however, the number of at least partially replicated findings is limited.[18] The statistical problem of type I errors (i.e., chance findings due to multiple comparisons) is particularly apparent in whole-genome association studies involving

many thousands of comparisons. Clearly, the identification and control of the factors leading to inflation of type I error will be instrumental to the future genetic studies in AD.

Ib. Genetics of sporadic AD

Sporadic AD accounts for the majority of all AD cases. Genetic factors seem to influence the risk for the development of sporadic AD 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 AD, most candidate genes are involved in amyloid metabolism, in brain cholesterol homeostasis or are components of the AD-related immune reaction. *APOE* is the only hitherto well-established risk factor for sporadic AD. Research findings on all the other genes remain controversial.

Apolipoprotein E (APOE) polymorphisms—Located on chromosome 19, the *APOE* gene encodes the apolipoprotein E, which is known to play a central role in the regulation of the cholesterol and triglyceride metabolism[19] and which has been more recently suggested to play a direct or indirect role in the development of AD pathology. There are three common *APOE* alleles, known as e2, e3, and e4. Each person can have any combination of these 3 alleles, resulting in the e2e2, e2e3, e3e3, e3e4, or e4e4 *APOE* genotypes. In comparison with the most common *APOE* e3e3 genotype, having an e2 allele is associated with a lower risk of AD and a slightly older median age at dementia onset. Even better established, each additional copy of the e4 allele in a person's *APOE* genotype is associated with a higher risk of AD and a slightly younger median age at dementia onset.

The *APOE* e2, e3, and e4 alleles are distinguished from each other on the basis of two SNPs, resulting in two amino acid changes at positions 112 and 158. The *APOE* e2-allele is characterized by cysteine at positions 112 and 158, the *APOE* e3-allele by cysteine at position 112 and arginine at position 158, and the *APOE* e4-allele by arginine at both positions.

A significant association of the *APOE* e4-allele with AD was initially demonstrated in 1993.[20;21] This finding was replicated in numerous studies and in now considered the best-established genetic association with AD. In Caucasian populations, *APOE* e4 heterozygous individuals have a threefold increased risk and homozygous persons at least an eightfold increased risk for developing AD by age 75 years compared to *APOE* e3 heterozygous individuals.[14] The magnitude of the effect of the *APOE* e4-allele as a risk factor for AD is influenced by a person's age and ethnicity. Although the *APOE* e4 effect is evident at all ages between 40 and 90 years, it becomes weaker after the age of 70 years. The highest odds ratios (ORs) are detected in the Japanese population (OR = 5.6 for *APOE* e4-allele heterozygous, OR = 33.1 for *APOE* e4-allele homozygous).[14] The *APOE* e4 effect is attenuated in Hispanic populations (OR = 2.5 for *APOE* e4-allele homozygous), and, interestingly, in some African populations no association between *APOE* e4 and AD can be observed.[22;23] These results together with the observation that the frequency of the *APOE* e4-allele in Pygmies, aborigines of Malaysia and Australia, Papuans, some Native Americans, and Lapps is significantly higher than in populations with long-established agricultural economy[24] indicate that the association between AD and the *APOE* e4 allele can be explained in part by the interaction of this allele with contemporary environmental conditions.

Due to the high ORs, at least in Caucasian populations, the possibility of using the *APOE* genotype as a diagnostic tool has been considered.[25;26] However, as stated by the American College of Medical Genetics/American Society of Human Genetics Working Group on *APOE* and Alzheimer disease,[27] *APOE* genotyping is not currently recommended to predict a healthy person's risk for AD, because the *APOE* genotype fails to predict with sufficient

certainty whether or when a person will develop symptoms.[28] However, if future treatments prove to be associated with a preferential response according to *APOE* genotype group, the possibility of disclosing genotype information to patients should be carefully evaluated. Indeed, randomized trials (i.e. the REVEAL study) are currently evaluating the benefits and risks of disclosing genotype information following a person's informed consent.[29;30]

Genetic testing—In accordance with the American College of Medical Genetics/American Society of Human Genetics Working Group on *APOE* and Alzheimer disease,[27] neither *APOE* nor any other susceptibility gene is currently recommended for diagnostic or prognostic genetic testing. However, when a person's family history is compatible with a Mendelian mode of inheritance, which is the case for the known *APP*, *PSEN1* and *PSEN2* mutations, genetic testing may be highly informative and useful. On the other hand, the deterministic character of genetic testing in such incurable diseases as AD may have far-reaching consequences for the patients and their families. In such cases, genetic testing requires a highly sensible and professional approach. Paralleling the guidelines for genetic testing in Huntington's disease,[31] genetic testing in AD must be accompanied by competent pre-and post-test counselling done by certified specialists.[32]

Additional risk genes for LOAD—Significant findings have been obtained for several additional genes, which may be involved in the AD-related pathophysiologic cascade, such as cholesterol 24S-hydroxylase (*CYP46A1*),[33] cystatin-C (*CST3*),[34] interleukin 1 (*IL1*),[35;36] and interleukin 6 (*IL6*)[37;38] and, very recently, apolipoprotein A1 (*APOA1*)[39] and ubiquilin 1 (*UBQLN1*).[40] Polymorphisms in these genes have been suggested as potential susceptibility factors for AD, which may also modify the onset of the disease. However, none of these findings has been consistently replicated thus far, which, among other factors, may be related to the current lack of systematic meta-analyses for each reported SNP in populations of different origin. By gathering data on published SNPs and association studies, the recently developed AlzGene database aims to provide an unbiased, publicly available and regularly updated collection of genetic association studies on AD (http://www.alzforum.org/res/com/gen/alzgene/). Although the integrated meta-analyses of this database are a very valuable tool, there is still no possibility to study gene-gene interactions, which undoubtedly exist in this polygenic disease.

New perspectives

Genetic clusters: Genetically complex phenotypes and diseases result from the interplay between genetic variants and environmental factors.[41] Despite this fact, the vast majority of the genetic association studies has either evaluated one genetic variation at a time or has analyzed multiple variations independently. However, this approach ignores the polygenic nature of the studied phenotype, focuses on marginal gene effects, inflates the probability of type I error, and erroneously assumes that the impact of the variation under study is comparable to a major gene effects. Recent developments in high-throughput genotyping and computerized algorithms now facilitate multi-locus analyses for the localization of complex trait genes. As shown for schizophrenia[42] and AD,[43] multi-locus analyses acknowledge the polygenic nature of disease, provide sets of trait-associated markers and also control for multiple testing. Importantly, such analyses may provide individual genetic clusters with potentially important clinical implications for future diagnosis, prognosis, and personalized treatments.

Genome-wide association (GWA): Candidate-gene association studies successfully identify genetic variations with significant impact on a polygenic trait. However, the success of such hypothesis-driven studies strongly depends upon pre-existing information, which limits their potential to identify novel genes and molecular pathways. Recent advances in the development of high-density genotyping platforms with the ability to genotype >500,000 of SNPs now allow

for whole-genome association studies in polygenic phenotypes and diseases. The highest genomic resolution reported so far corresponds to 1 SNP per 26 kb (i.e. GeneChip® Human Mapping 100K Set). Although the current statistical approaches are not ideal for the analysis of the huge amount of data, the first genome-wide association studies already revealed highly reliable and novel genetic findings.[44] Therefore, GWA is expected to substantially increase our knowledge on polygenic human traits.

AD endophenotypes: To further evaluate genetic and non-genetic modifiers of the risk for a neuropsychiatric disorder like AD, it may be possible to identify an "endophenotype," i.e., a measurable feature that is more closely related to disease susceptibility than the clinical syndrome itself.[45;46] For instance, patients with AD have characteristic and progressive declines in positron emission tomography (PET) measurements of the cerebral metabolic rate for glucose (CMRgl) in the posterior cingulate, parietotemporal, and frontal cortex. Studying 160 cognitively normal persons with two copies, one copy, and no copies of the APOE e4 allele, Reiman et al. recently showed a significant correlation between these three levels of genetic risk for AD and lower CMRgl in each of these brain regions. They have suggested how PET could be used as a quantitative, presymptomatic endophenotype to help evaluate individual and aggregate effects of putative genetic and nongenetic modifiers of AD risk.[46] Brain imaging techniques like PET, and other biomarkers of AD susceptibility could complement retrospective case-control studies and prospective cohort studies in the evaluation of these putative modifiers of AD risk.

Ic. Genetics of autosomal-dominant AD

In some families, AD 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 AD). These rare AD families have been of utmost importance for the identification of causative AD genes by using the methods of linkage analysis with subsequent positional cloning. This strategy has led to the identification of 3 hitherto known AD-related genes.

Amyloid precursor protein (APP)—In 1987, St. George-Hyslop et al.,[47] located a genetic defect causing autosomal-dominant AD on the long arm of chromosome 21. The *APP* gene, which codes for the amyloid precursor protein, was found to map to this region.[48] Interestingly, a mutation in exon 16 of *APP* was found to cause hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D).[49] HCHWA-D is associated with Aβ deposition in cerebral blood vessels with the consequence of recurrent cerebral hemorrhages. Moreover, amyloid plaques similar to those found in AD 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 AD would be very high. In 1991, Goate et al. described the first missence mutation in exon 17 of *APP* co-segregating with familial AD.[50] Subsequent studies identified additional *APP* mutations in families with presenile AD. 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 AD, their effect may be additionally modified by gene-gene interactions: In the majority of families bearing *APP* mutations, the *APOE* e4 allele results in an earlier age at onset.

The identification of *APP* mutations was instrumental for the understanding of the metabolic cascades leading to enhanced Aβ production and gave rise to the amyloid hypothesis of AD.[51] However, only a small percentage of autosomal-dominant AD 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 AD was identified on the long arm of chromosome 14 by Schellenberg et al. in 1992.[52] 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 missence mutations cosegregating with early-onset autosomal dominant AD.[53] Since then, several *PSEN1* mutations have been identified, often resulting in an aggressive, early form of the disorder between ages 35–65 years. Thus, *PSEN1* mutations account for the majority of autosomal-dominant AD cases (Table 2). Most presenilin mutations are missence 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.[54] 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.[55–61] 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 AD gene, the mechanism by which mutations of this gene caused the AD phenotype was an open matter and was 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.[62] 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. Presenilins are currently considered to be the catalytic subunits of the γ-secretase complex, which also contains three additional subunits (Nicastrin, Aph1, and Pen-2).[63]

Presenilin 2 (PSEN2)—In 1988, Bird et al. described a group of families with autosomal-dominant early-onset AD descending from a single German family that first immigrated to Russia and later to the United States (‘founder effect’).[64] No *APP* or *PSEN1* mutations were detected in these Volga-German kindreds. The search for proteins homologous to presenilin1 led to the cloning and characterization of the *PSEN2* gene, which is located on chromosome 1q31-q42 and codes for a protein highly homologous to presenilin 1.[65] Ten missence PSEN2 mutations have been described so far (Table 3). 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.

Id. Conclusion

AD 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 AD 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 characterization 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 dechiphering 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 optimization of therapeutic strategies.

II. Transcriptional Profiling in AD

Genetic variability may be considered as the first source of phenotypic variability between individuals. However, the limited number of genes in the human genome (approximately 30000) suggests that a significant portion of interindividual differences is related to modifications at the transcriptional, translational, and post-translational level. The high-throughput analysis of gene transcripts ("transcriptomics"), which allows for the simultaneous assessment of thousands of mRNAs, is a valuable approach to identify genes that are differentially expressed in certain conditions.

Gene expression microarrays provide a powerful tool for understanding the alterations in cellular metabolism that occur during the course of AD and for developing targeted therapeutics to correct those alterations. The technology has, in the past several years, stabilized to the point where robust expression correlates of clinical traits can be ascertained, validated, and used to gain insight into the pathogenesis of a disorder. Numerous studies report specific gene dysregulation events associated with AD and have suggested important mechanisms whereby neuronal cells die in this disorder. More recent advances in single cell approaches to gene expression profiling allow the analysis of pathologically affected cell types specifically rather than of the heterogeneous brain sample as a whole and should allow more focused interrogation of disease mechanisms, thereby permitting development of therapeutics targeted specifically to pathologically affected cells.

There are two primary variations of expression arrays currently in use, cDNA and oligonucleotide microarrays. Oligonucleotide arrays provide the benefits of greater specificity since the probes used are of shorter sequence (~25–70 nucleotides) than those used for cDNA arrays (200-2000 nucleotides). cDNA arrays have greater sensitivity but cannot, for example, discriminate between splice variants because the size of DNA probes used often spans splice junctions. For each type of array, sequences of DNA that are homologous to different genes of interest are attached to a solid support at distinct locations. Each probe (or probe set for oligonucleotide arrays with built in redundancy such as the Affymetrx platform) on a slide corresponds to a single gene and each array can hold thousands of probes. One then hybridizes either labeled cRNA or cDNA from the cell type or tissue of interest to the microarray, rapidly generating an mRNA expression profile for thousands of genes.

Analysis of expression profiling data entails comparison of profiles from the state of interest to control profiles using statistical strategies which fall into two general categories: supervised and unsupervised approaches. Supervised analysis entails specifying the disease class before analysis proceeds. This approach allows generation of a list of genes whose expression differs significantly between the phenotypic classes of interest. Unsupervised approaches do not specify a phenotypic class a priori, rather allow similarity measures to be derived between expression data based strictly on the expression values, and phenotype is then superimposed on the arrays. Expression array analysis, while not completely straightforward, has matured to the point where the technology can be used as a robust screening tool and thus derive differences in gene expression which can then move forward into validation in independent sample sets at the protein level.

IIa. Whole tissue expression profiling in Alzheimer's disease

Numerous expression microarray experiments to date have identified specific genes that are dysregulated under different AD-related conditions. The general conclusions from these studies are that there is a general downregulation of gene expression in the cortices of AD affected brains compared to unaffected individuals. Because each microarray experiment will identify over one hundred genes which are dysregulated between control and AD it is impossible to list all of the correlations that have been identified within the context of this review. Rather, we highlight several findings that have generalized significance to the pathological processes of AD. For example, alterations of the amyloid precursor protein, inflammatory pathways, and synaptic signaling components have been reported.[66–68] All of these expression profiling results fit well with known pathological lesions of AD and provide interesting gene candidates that may underly these processes. Using cDNA microarrays, Ho *et al*. demonstrated the selective decrease in expression of synapsin-a splice variants I–III in the entorhinal, but not visual, cortex of post-mortem brains of early stage AD patients.[66] Thirty-two genes were found to be differentially expressed ≥ 1.8-fold in samples from patients displaying moderate dementia compared to normal controls. Colangelo *et al*., using oligo-based microarrays on pooled hippocampal CA1 tissues from six individuals, demonstrated a generalized down-regulation in brain gene transcription, including decreases in RNA encoding transcription factors (ISGF, GATA-2, LYL-1 and GATA-4) as well as neurotrophic factors and synaptic signaling elements (synaptophysin, SLIT-2, NCP, CHAT, metallothionein III, and metal regulatory factor-1), which fits well with the observed synaptic dysfunction that occurs in AD.[67] Expression increases in the βAPP gene were demonstrated along with increases in pro-apoptotic genes (DAXX, CDP5, and FAS) as well as pro-inflammatory genes ($cPLA_2$, NF-κBp52/p100, DPP1, NFIL6, IL-1β, B94, HB15, COX-2, and CEX-1). Another group examined brain tissue from a single AD patient (hippocampal tissue containing neurofibrillary tangles vs. parietal cortex with no lesions) and showed a dramatic increase in the expression of the calcineurin Aβ gene.[68] This group found a total of 310 differentially expressed genes, of which, 20 up- and 20 down-regulated were chosen for validation by *in situ* hybridization and RT-PCR. Calcineurin Aβ (protein phosphatase 2B, a serine/threonine phosphatase), a major calmodulin-binding protein in the brain was the most highly up-regulated gene identified. Its proposed mechanism of involvement in AD pathogenesis is its ability to dephosphorylate hyperphosphorylated tau protein.

More recent microarray experiments have focused on characterizing gene dysregulation in animal models of AD. In some cases, introduction of pathogenic mutations in the genes encoding APP, the presenilins, and tau into mice results in the development of AD pathologies, such as amyloid plaques and neurofibrillary tangles. Expression profiling has been used to identify the downstream effects of these pathogenic mutations in these transgenic animal models of AD.[69–74] Specifically, analysis of PS-1 knockout mice revealed upregulation of inflammatory pathways, including complement component C1q.[69] PS1 knockout and PS1 knock-in (DeltaE9 mutation) models of AD have also been directly compared.[73] In this study, the PS1 knockout and mutant form of PS1 had opposite effects on the expression of specific genes, leading the authors to conclude the this particular pathogenic PS1 mutation may be a gain-of-function mutation. Analysis of APP/PS-1 (P264L) transgenic mice, which develop amyloid plaques, revealed upregulation of the immune response, carbohydrate metabolism, and proteolysis as well as downregulation of growth factor pathways (BDNF and IGF-1R).[71] Each of these studies provides additional candidate genes that may mediate some of the known pathological hallmarks of AD, including the deposition of amyloid plaques, the aggregation of tau protein into neurofibrillary tangles, and the activation of glial cells in AD affected brain regions. The challenge now is to develop the appropriate functional assays and endpoints to determine the specific role that dysregulation of these genes plays in the development and progression of AD.

IIb. Single cell expression profiling in Alzheimer's disease

All of the expression profiling experiments summarized above suffer from the major limitation of utilizing heterogeneous brain tissue samples, each of which are composed of at least half a dozen distinct neuronal and glial cell types and numerous neuronal subtypes. This adds significantly to the variability of these experiments and increases the likelihood that significant and pathological gene expression changes may be masked by the expression profiles of additional unaffected cell types. Such a masking would result if the cell types of interest are in the minority relative to the piece of whole brain used in the expression profiling experiment. Recent advances in microaspiration and microdissection techniques, in RNA amplification procedures, and in banking of high quality, clinically well characterized tissue now afford the opportunity to investigate the expression of specific pathologically affected cell types in clinically well-stratified patient cohorts to identify the full complement of gene expression changes that accompanies the pathological progression of Alzheimer's disease.

To date, the application of single-cell expression profiling techniques to Alzheimer's disease has been somewhat limited and includes an analysis of anterior nucleus basalis neurons from control and AD brain[75] and characterization of expression changes associated with neurofibrillary tangle pathology in AD.[76] These studies suggest important roles for the upregulation of neurotrophic signaling and downregulation of protein phosphatase activity in nucleus basalis neurons.[75]

Large scale efforts are currently underway to characterize the full complement of neuronal and glial gene expression changes that occur during the course of AD. This information will provide novel genes and mechanisms whereby neuronal cells are lost in AD. Clearly the ultimate goal of these expression studies is to identify the gene targets that can be used to develop novel and effective treatment strategies. Within our own groups, we have begun a comprehensive cataloging of neuronal malfunction relative to controls in a mild cognitive impairment patients as well as AD patients in six regions of the brain. We feel that looking at early stage disease will allow insights to be gleaned with respect to the early pathogenic cascades at work, and thus allow us to eventually design therapeutics aimed at the earliest cellular events leading to neuronal death. Initial findings from this effort highlight diverse molecular pathways associated with NFT formation in the entorhinal cortex during AD.[77]

IIc. The Future of Expression Profiling in Alzheimer's Disease

The ultimate goal of the application of expression profiling technology to AD is to identify effective therapeutic targets, to assist in the development and testing of new drugs that hit those targets, and further, to identify which individuals are likely to respond to therapy. The realization of this goal depends, however, on the understanding of the experimental challenges and on the implementation of appropriate validation steps which complement expression studies. Indeed, different data mining methods, computational algorithms, and genes used for normalization are some of the numerous variables which influence an experiment's outcome and reduce the comparability between studies.[78;79] In addition, if the transcriptomics approach is limited only to cross-sectional comparisons, the identification of genes which represent rather a consequence, compensation or coincidence rather than the cause of the disease is likely. Therefore, appropriate corroborating procedures such as the analysis of specific cell types and the targeted manipulation of genetic pathways should be included in studies dealing with the expression pattern of several thousands of genes.

As advancement of single cell methods, coupled to ever-improving animal model outlets for functional validation of gene candidates proceeds, expression profiling in AD will move from an exploratory tool to interrogate disease mechanisms to an essential component in the development of safe and efficacious treatments. For example, a recent report uses expression

profiling to track the changes associated with inhibition of CDK5, a gene thought to be important in the hyperphosphorylation of tau protein.[80] Using this approach, the authors were able to show that the CDK5 inhibitor altered the expression of many genes involved in the pathological processes associated with Alzheimer's disease and neuronal cell death, including numerous genes involved in synaptic transmission, neurite outgrowth, and neuronal survival.

Increasingly, as the application of microarrays to reveal disease mechanisms leads to the development of new drugs to slow or reverse those mechanisms, the focus of microarray technology will shift to analyzing therapeutic effectiveness of those drugs. The availability of a real-time monitoring system to track the molecular effects of therapy may be particularly useful for AD, where important molecular changes may precede clinical manifestation of disease by decades. Additionally, this approach may be increasingly useful in a healthcare environment where unforeseen drug side-effects could shelf any potentially useful drug at any time. Knowledge of the full molecular effects of a given drug may provide a harbinger for side-effects that would otherwise have passed current regulatory standards, thereby sparing the expense of withdrawing an otherwise useful pharmaceutical from the market. There has been little application of state-of-the-art expression profiling technologies to AD, but a multitude of studies are currently underway which should allow insight into the somatic changes in the brains of AD patients.

III. Proteomics in Alzheimer's disease

Proteomics is a multidisciplinary, technology-driven science that is focused on the analysis of proteomes: i.e. the proteins of a biological system, their structures, interactions, post-translational modifications, and in particular the changes in their levels and their modifications, which are the result of specific diseases or are induced by various external factors, such as toxic agents. The goal of proteomics is to detect novel drug targets and diagnostic markers. Proteomics emerged during the last few years, and its development can be attributed to developments in mass spectrometry, computer and software sciences and to the enormous amount of information that became available from the sequencing of the genome of many organisms. Proteomics can detect gene products involved in various pathways which can not be detected in another way and its major difference from the previously existing protein analytical techniques is that proteomics does not analyze the proteins one by one, but in a possibly automated, large-scale mode.[81]

A proteomic analysis comprises two steps: (i) separation of the protein mixture in order to allow the efficient detection of the particular proteins included in the mixture and (ii) identification of the separated proteins by various analytical methods, mainly by mass spectrometry (MS). Separation is usually performed by two-dimensional (2-D) gel electrophoresis or liquid chromatography (LC) or more often by combination of the two approaches.[82–84] Two-dimensional electrophoresis is the principal separation method in a proteomic analysis and has the advantage that it allows the simultaneous detection of thousands of proteins. It comprises two steps (dimensions), separation of the proteins on the basis of differences in their net charge by isoelectric focusing (IEF), and separation of the focused proteins on the basis of differences in their molecular masses by sodium dodecyl sulfate (SDS) polyacrylamide gels.[85;86] When liquid chromatography is applied for protein separation, the intact proteins can be first fractionated by ion exchange and/or hydrophobic interaction chromatography or by a combination of various binding principles. In a next step, the proteins of the fractions collected are digested and the peptides are separated by reversed phase HPLC and analyzed by MS.

The most efficient and most widely used protein identification method in proteomics is the peptide mass fingerprint (PMF) approach which is mainly performed by matrix-assisted laser

desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF-MS).[87] For MS analysis, protein spots are excised from 2-D gels, the proteins are in-gel digested, the digests together with the matrix are applied on the sample target and are measured in the mass spectrometer.[88] In a high-throughput proteomic analysis, most of the operations are performed in automated mode and several thousands of protein spots can be analyzed by one person per day. The analysis of certain groups of proteins is more favorably performed by LC/MS, e.g. very acidic/basic, small proteins/peptides, and hydrophobic proteins. However compared to 2-D gels, isoforms, post-translational modifications, and processing products are very difficult to discern via LC/MS.[89] Fig. 1 shows the workflow for the proteomic analysis of the brain, involving preparation of protein subfractions, protein separation by 2-D electrophoresis or LC and identification by mass spectrometry.

Proteomics is routinely used in clinical diagnosis today, and it has been applied in the investigation of infectious diseases, cancer, heart, and neurological disorders. The applications are summarized in several review articles.[81;89–94] In the study of AD, proteomics has been mainly used for the analysis of human brain, of the brain of animals serving as models of AD and of cerebrospinal fluid (CSF) from patients with AD with the goal to collect information about gene products involved in the diseases i.e., alterations in protein levels and post-translational modifications.[89] Proteomics has also been used in the investigation of Down syndrome (DS) and other neurodegenerative disorders. Almost all subjects with DS over 40 years show neuropathological and neurochemical abnormalities on post-mortem brain examinations that are indistinguishable from those seen in Alzheimer's disease. Thus, results from the study of the DS brain may be useful in the AD studies.[95–98]

IIIa. Proteins with deranged levels and modifications in AD

Most neuroproteomics analyses involve 2-D electrophoresis and MALDI-TOF-MS steps, as this approach allows for a reliable quantification of changes in protein levels. Fig. 2 shows the proteins of total brain extract separated on a 2-D gel. The most abundant proteins detected in 2-D gels carrying total brain extract are structural proteins, like tubulin chains and actin, heat shock proteins, dihydropyrimidinase-related proteins-2 and -3, and house-keeping enzymes. The less abundant proteins are mainly hypothetical proteins, enzymes, as well as structural proteins. The relative levels of the low-abundance brain proteins vary in the range 0.005–0.01% and of the high-abundance about 3% in comparison with the levels of total proteins detected in the corresponding gel.[89] In one study, the LC-MS/MS approach was used for the detection of proteins enriched in amyloid plaques in the AD brain.[99]

The levels of about 100 proteins were found to be changed in the AD brain and cerebrospinal fluid in comparison with the control brain and CSF, respectively. The observed changes in the protein levels usually vary in the order of 20–100% in comparison with the average levels of the control group. The largest changes were observed for the glial fibrillary acidic protein (GFAP), which distinguishes astrocytes from other glial cells during the development of the brain. For this protein, ten-fold or even higher levels were observed in individual AD brains, compared to the average levels of the control group.[100] Fig. 3 shows an example of a protein, synaptosomal protein 25 kDa (snap25), which shows reduced levels in the AD brain in comparison with the control brain. The proteins detected by proteomics approaches to show changed levels or modifications in AD have various functions, mainly involved in conformational changes, neurotransmission, guidance, signal transduction, metabolism and detoxification. The CSF proteins with deranged levels are plasma proteins. The proteins detected by proteomics methods to have deranged levels in the AD brain and the CSF are listed in Table 4.

Heat shock proteins (HSP) are involved in conformational changes of the brain proteins, i.e. in protein folding and assembly into oligomeric structures.[101;102] Several heat shock proteins

showed deranged levels in the AD brain. Increased expression was observed for alpha crystallin B, glucose regulated protein 94 (GRP 94) and HSP70 RY, and decreased expression for heat shock cognate (HSC71), GRP 75, HSP60 and T-complex protein-1.[103] The inconsistency in the direction of change of the heat shock proteins in AD may be explained by the different response of the individual heat shock proteins to stress conditions. Increased expression of heat shock proteins in AD may represent a defensive mechanism of response to amyloid fibril formation, as chaperone proteins are capable of preventing aggregation of other proteins and in particular inhibiting self-assembly of polyglutamine proteins into amyloid-like fibrils. Reduced levels of the HSP70 proteins, in particular of the HSC71, might be linked to their protective role in neuronal death associated with AD. HSC71 may be involved in the structural maintenance of the proteasome and conformational recognition of misfolded proteins by proteases.[104;105] Thus, decreased activity of HSC71 which is attributed to either reduced levels[103;106] or increased oxidative modification of the protein[104] may be responsible for impaired protein clearance and deposition of amyloid-β peptide in the AD brain.

Synaptosomal proteins, soluble N-ethylmaleimide sensitive factor attachment proteins (SNAPs) forms α, β, γ, synaptososmal associated protein 25 (SNAP 25), synaptotagmin and vesicular proteins, are involved in synaptic transmission which is deranged in AD. Proteomics studies showed reduced expression for SNAP 25[100] (Fig. 3), β-SNAP and synaptotagmin I[107] in the AD brain and suggest impaired neurotransmission in AD.

Guidance is facilitated by proteins signaling the correct path to the growing axon. Dihydropyrimidinase-related protein 2 (DRP-2), also known as collapsin response mediator protein-2, is a cytosolic protein that shows a high homology to the rat turned on after division 64 kDa protein and the chicken collapsin response mediator protein-62, which are involved in path finding and migration of neurons. The functions of DRP-2 are not well known. It is possibly involved in neuronal repair and interacts with and modulates the activity of collapsin, a protein that elongates dendrites and directs them to adjacent neurons. Proteomics studies revealed deranged expression levels[108] and oxidative modification[104] of DRP-2 in the AD brain. The reduced levels of DRP-2 in the AD brain may be responsible for synaptic loss in AD as it can not respond to the formation of new synapses. Moreover, DRP-2 is immediately modified by oxidation and looses activity, which results in shortened dendrites that would cause decreased interneuronal communication and memory impairment.[109]

The levels of several signal transduction proteins have been found to be deranged in the AD brain. Thus, reduced levels were found for 2′, 3′-cyclic nucleotide-3′phosphodiesterase (CNPase), [110] nucleoside diphosphate kinase (NDK)-A[111] and stathmin[112] and increased expression for 14-3-3 gamma and epsilon proteins.[113] CNPase is associated with oligodendroglia and myelination. NDKs are oligomeric enzymes, involved in the maintenance of the intracellular pool of deoxynucleotide triphophates and nucleotide triphosphates, playing regulatory roles in the activation of G-proteins.[114] Stathmin, a major substrate of cyclin-dependent kinases and protein kinase A, is involved in multiple signal transduction pathways. Stathmin possibly plays a role in neurodegeneration and impaired signaling because of its negative correlation with neurofibrillary tangles, a hallmark of AD neuropathology, and interaction with tubulin to cause microtuble destabilization.[112] 14-3-3 proteins form homo- or heterodimers and are primarily, but not exclusively expressed in neurons and bind to and modulate the function of a wide variety of cellular proteins. They are involved in neuronal development, signal transduction, cell growth and cell death. They show increased levels in AD which may be rather linked to apoptosis than to other cellular activities, as they are highly enriched in areas of massive neuronal death caused by pathological processes.[115] The increased levels of 14-3-3 proteins in AD may represent a protective response against apoptosis.

Oxidative stress, either detected as protein oxidation, lipid peroxidation, DNA oxidation and 3-nitrotyrosine formation, is considered to be a common mechanism for different age-related neurodegenerative pathologies. The levels of antioxidant proteins in the brain were studied using proteomics technologies. Superoxide dismuatase (CuZn, SOD1) catalyzes the dismutation of superoxide anion into hydrogen peroxide. The protein showed decreased levels in AD and the decrease may reflect cell loss in AD.[116] The expression of two other proteins, carbonyl reductase and alcohol dehydrogenase, involved in oxidative stress, were found to be increased in AD.[117] These two cytosolic enzymes catalyze reduction of carbonyls, which are toxic metabolic intermediates, increased in the AD brain[104;105] and serve as markers for oxidative stress, suggesting a possible role of oxidation-related decrease in protein function in the process of neurodegeneration. The increased levels of these enzymes may represent an adaptive mechanism to detoxify the toxic intermediates. Peroxiredoxin I and II showed increased and peroxiredoxin III decreased levels in AD brain.[118;119] Peroxiredoxins are a family of peroxidases that protect biomolecules by reducing hydrogen peroxide and alkyl hydroperoxides. There is evidence that over-expression of peroxiredoxins, in particular of peroxiredoxins I and II, in various cell lines abrogates the effects of agents that positively modulate apoptosis, including hydrogen peroxide.[120] Enhanced apoptosis has been demonstrated in AD[121] and up-regulation of these enzymes could represent a cellular response against apoptosis. In other neurodegenerative disorders, like DS and Pick's disease, deranged levels for peroxiredoxins were observed as well.[118;119]

Oxidative stress is observed in the AD brain in regions where amyloid β-peptide 1-42 is present, but not in cerebellum where AD pathology is not observed.[122] Several oxidized proteins were detected by immunoblots in the AD brain and identified by MALDI-TOF-MS (Table 4). One of the oxidized proteins is creatine kinase (BB isoform). Amyloid β-peptide inhibits creatine kinase and causes lipid peroxidation leading to decreased energy utilization, alterations in cytoskeletal proteins and increased excitotoxicity, all reported for AD. The other oxidized proteins are glutamine synthase, which is related to increased excitotoxicity, ubiquitin C-terminal hydrolase L-1, associated to aberrant proteasomal degradation of damaged or aggregated proteins, α-enolase, linked to altered energy production and dihydropyrimidinase-related protein 2, related to reduced growth cone elongation.[122;123] The ubiquitin C-terminal hydrolases are suggested to hydrolyze small adducts of ubiquitin and generate free monomeric ubiquitins, thereby allowing recycling of ubiquitin, which is essential to the function of the proteolytic machinery. Thus, putative dysfunction of this oxidized deubiquitinating enzyme may be one way by which oxidative stress promotes ubiquitin-positive inclusion biogenesis, a pathological characteristic of the AD brain, which leads to neurodegeneration.

Using LC-MS/MS, Liao et al., identified 26 brain proteins enriched in amyloid plaques excised with laser capture microdissection from AD brains.[99] The list includes cytoskeletal proteins (coronin, tau), membrane trafficking proteins (clathrin, dynamin 1, dynein), proteases (ATPases, cathepsin), signal transduction proteins (14-3-3 proteins), chaperons and others (Table 4).

IIIb. Limitations

The proteomic analysis of the brain has certain limitations which can be related to the sample and/or the analytical approach. In the analysis of the brain, many factors may be involved, such as differences among individuals, differences in age and sex, possibly other diseases, treatment with medicines, as well as technical, disease-unrelated factors, such as post-mortem time, improper treatment of the samples etc., all of which can affect a clear discrimination between healthy and diseased states of interest. The technical limitations involve inefficient detection of low-abundance gene products, of hydrophobic proteins (they do enter the IPG strips), acidic, basic, high- and low-molecular mass proteins. All these protein classes are underrepresented

in 2-D gels.[89;124] A combination of the proteomics methods with protein separation and enriching techniques and alternative methodology for detection will improve the detection of additional differences between AD and control brain. Such differences may be essential in the discovery of early disease markers and therapeutic approaches.

IV. Conclusions

AD is a genetically heterogeneous disorder. The recent advances in the detailed characterization of the human genome, the identification of millions of polymorphic sites in the human DNA and the development of high-throughput genotyping methods and elaborated statistical analyses will soon contribute to the identification of genetic risk profiles related to the development and course of this devastating disease. Additional, highly informative and relevant methods such as high-throughput gene and protein expression studies are now available. The intelligent integration of knowledge derived from genetic, transcriptomic and proteomic studies will greatly advance our understanding of the causes of AD, it will improve our capability of establishing an early diagnosis, it will help defining disease subgroups and it will ultimately help to pave the road towards improved and tailored treatments.

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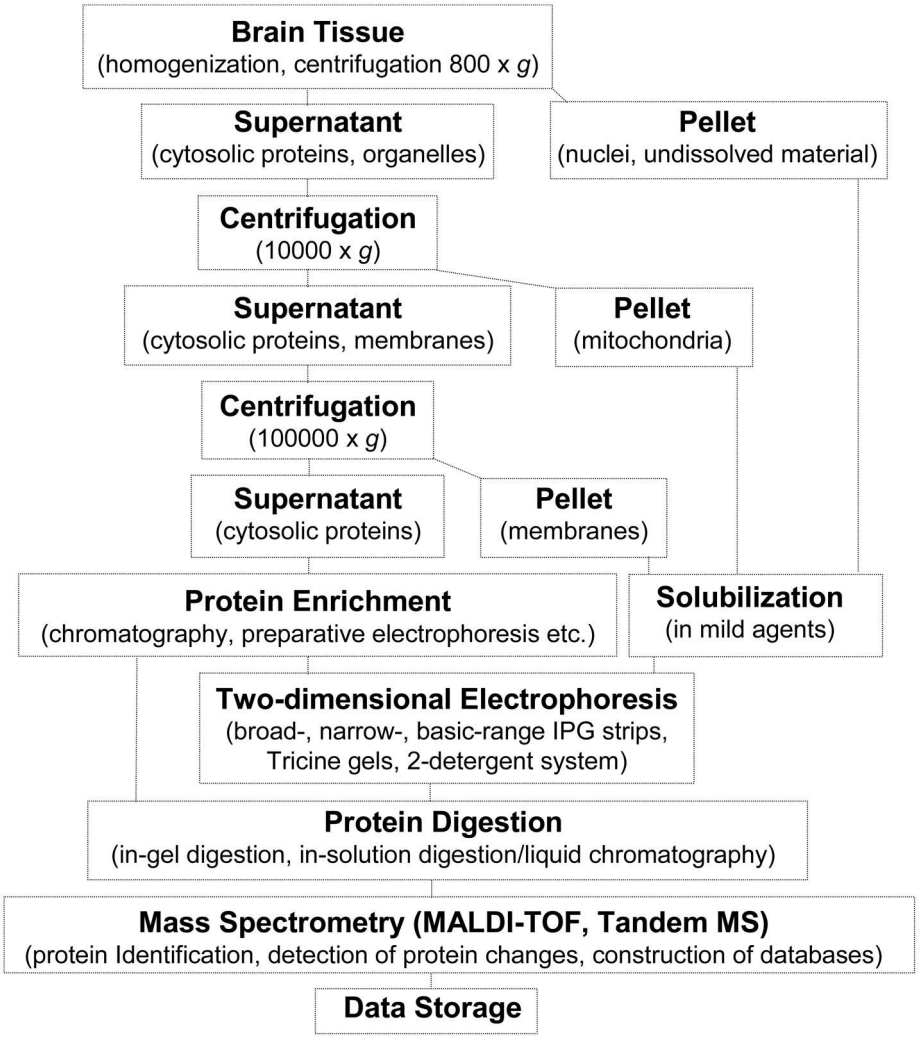

Figure 1.
Workflow for the proteomic analysis of the brain.

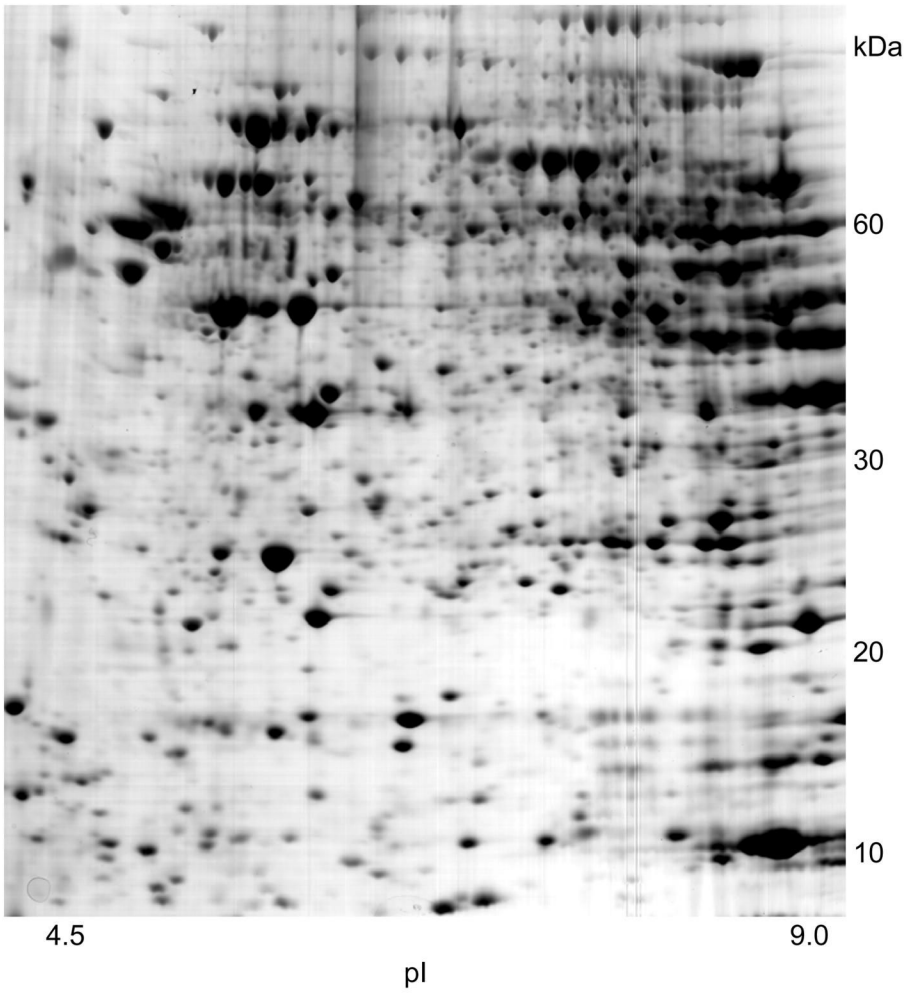

Figure 2.
Proteins of total brain extract separated on a 2-D gel.

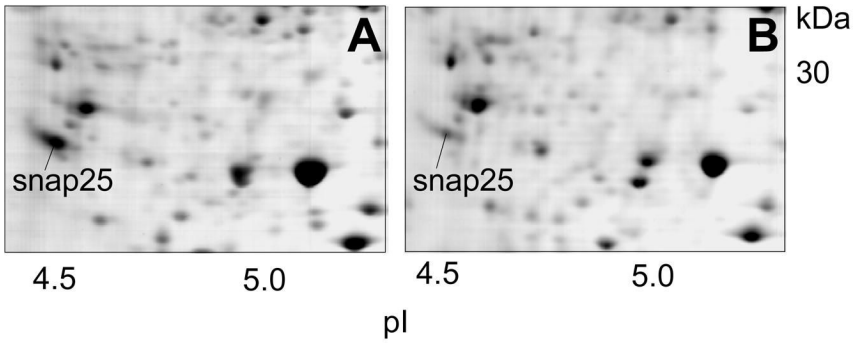

Figure 3.
Example of a protein, in this case the synaptosomal protein 25 kDa (snap25), which shows reduced levels in the AD brain in comparison with the control brain.
A: controls brain, B: AD brain.

Table 1

Pathogenic *APP* gene mutations

Exon	Mutation	Citation
16	K670N	125
16	M671L	125
16	D678N	126
17	A692G	127
17	E693Q	49
17	E693G	128
17	D694N	129
17	A713T	130;131
17	T714A	132
17	T714I	133
17	V715M	134
17	V715A	135
17	I716V	136
17	V717I	50
17	V717F	137
17	V717G	138
17	V717L	139
17	L723P	140

Table 2

Pathogenic *PSEN1* gene mutations

Exon	Mutation	Citation
4	R35Q	141
4	A79V	143
4	V82L	145
4	Δ I83/M84	147
4	L85P	149
4	V89L	150
4	C92S	152
4	V94M	154
4	V96F	156
4	F105L	157
5	L113P	159
5	IVS4 g.23024delG	161
5	Y115H	145
5	Y115C	143
5	T116N	164
5	T116I	165
5	P117L	166
5	P117S	167
5	P117R	169
5	E120K	171
5	E120D	61
5	E120D	173
5	K123E	174
5	N135D	176
5	M139V	177
5	M139T	145
5	M139I	177
5	M139K	142
5	I143F	144
5	I143T	146
5	M146L	148
5	M146L	141
5	M146V	151
5	M146I	153
5	M146I	155
5	M146I	141
5	T147I	158
5	L153V	160
5	Y154N	162
5	Y154C	163
5	g.25669_25670 insTTATAT	141
6	H163Y	151
6	H163R	53
6	W165C	158
6	L166R	168
6	L166P	170
6	ΔI167	163
6	S169L	172
6	S169P	57
6	L171P	175
6	L173W	158
6	L174M	178
6	F177L	141
6	F177S	141
6	S178P	141
6	G183V	180
7	E184D	182
7	G206S	141
7	G206A	141

Exon	Mutation	Citation
7	G206V	185
7	G209V	173
7	G209R	186
7	G209E	141
7	I213T	156
7	I213L	141
7	I213F	169
7	G217D	191
7	L219P	193
7	Q222R	141
7	Q222H	179
7	L226R	196
7	I229F	163
7	A231T	145
7	A231V	143
7	M233T	198
7	M233L	200
7	M233V	202
7	L235P	145
7	L235V	163
7	F237I	204
7	F237L	163
7	A246E	53
7	L250S	171
7	L250V	207
7	Y256S	179
8	A260V	181
8	V261F	141
8	L262P	183
8	C263R	184
8	C263F	163
8	P264L	145
8	P267S	151
8	P267L	187
8	R269G	188
8	R269H	189
8	L271V	190
8	V272A	192
8	E273A	194
8	T274R	141
8	R278K	195
8	R278I	197
8	R278T	198
8	E280A	151
8	E280G	151
8	L282R	199
8	L282V	201
8	P284L	203
8	A285V	181
8	L286V	53
	Δ9	205
	Δ9	198
	Δ9	206
	Δ9	203
10	InsR	141
10	T354I	141
10	R358Q	141
10	S365Y	141
11	R377M	163
11	G378E	208
11	G378V	163

Exon	Mutation	Citation
11	G384A	146
11	S390I	158
11	L392V	181
11	L392P	209
11	G394V	141
11	N405S	210
11	A409T	200
11	C410Y	145
12	L418F	141
12	L424R	211
12	A426P	173
12	A431E	141
12	A431V	212
12	A434C	213
12	L435F	141
12	P436S	214
12	P436Q	172
12	I439V	141

Table 3

Pathogenic *PSEN2* gene mutations

Exon	Mutation	Citation
4	R62H	215
5	T122P	157
5	S130L	152
5	N141I	65
5	V148I	216
7	Q228L	169
7	M239I	157
7	M239V	181
12	T430M	217
12	D439A	217

Table 4

Altered proteins in AD

Protein	Change	Reference
Heat Shock Proteins		
Alpha crystallin B chain	I	103;218
Glucose regulated protein 75 kDa (GRP75)	D	103;218
Glucose regulated protein 75 kDa (GRP94)	I	103;218
Heat shock 60 kDa, mitochondrial	D	219
Heat shock 90kDa protein 1, beta	Enriched in amyloid plaques	99
Heat shock cognate 71	Oxidation	104
Heat shock protein 70 RY	I	103;218
T-complex protein 1 (TCP-1)	D	220
Synaptosomal proteins		
Beta-soluble N-ethylmaleimide-sensitive factor attachment protein (Beta SNAP)	D	107
Synaptosomal associated protein 25 kDa (Snap-25)	D	100
Synaptotagmin I	D	107
Guidance Proteins		
Didyropyrimidinase-related protein 2 (DRP-2)	D	108
Signal Transduction Proteins		
14-3-3 beta/alpha	Enriched in amyloid plaques	99
14-3-3 epsilon protein	I	89;113
14-3-3 gamma protein	I	89;113
14-3-3 zeta	Enriched in amyloid plaques	99
2′-3′-Cyclic nucleotide 3-phosphodiesterase (CNPase)	D	110
Alpha-Endosulfine	D	221
ARPP-19	D	222
cAMP-dependent protein kinase (PKA)	D	222
Fatty acid-binding protein, heart	D	219
Guanine nucleotide-binding protein beta subunit	D	219
Histamin-releasing factor	D	223
Nucleoside diphosphate kinase A (NDKA)	D	111
Stathmin	D	112
Antioxidant proteins		
Alcohol dehydrogenase (ADH)	I	117
Antioxidant protein 2 (1-Cys peroxiredoxin) (1-Cys Prx)	I	224
Carbonyl reductase (CBR)	I	117
Peroxiredoxin I (Prx-I)	I	118
Peroxiredoxin II(Prx-II)	I	118;119
Peroxiredoxin III (Prx-III)	D	119
Peroxiredoxin VI	I	119
Superoxide dismutase	D	116
Oxidized Proteins		
Alpha-enolase	Oxidation/Nitration	104
Beta-actin	Oxidation/D/Nitration	122;123
Creatine kinase BB	Oxidation/D	105;225
Didyropyrimidinase-related protein 2	Oxidation/D	104
Gamma-Enolase	Oxidation/Nitration	104
Glutamate Transporter, EAAT2	Oxidation/D	123
Glutamine synthase	Oxidation/D	105
Heat shock cognate 71	Oxidation	104
L-Lactate dehydrogenase	Oxidation/Nitration	104
Neuropolypeptide h3	Oxidation/Nitration	104;123;226
Triosephosphate Isomerase	Oxidation/Nitration	122;226
Ubiquitin carboxy-terminal hydrolase L-1	Oxidation	105
Metabolism, Memory		
ATP synthase alpha chain, mitochondrial	D	219
ATP synthase beta chain, mitochondrial	I	219
Beta-enolase	D	219
NADH-ubiquinone oxidoreductase 24 kDa subunit, mitochondrial [Precursor]	D	227
NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial [Precursor]	D	227
Phosphofructokinase, muscle ttype	Enriched in amyloid plaques	99
Phosphofructokinase, platelet type	Enriched in amyloid plaques	99
Ubiquinol-cytochrome-c reductase complex III core protein I	D	228
Voltage-dependent anion-selective channel protein 1 (VDAC1)	D	229

Protein	Change	Reference
Voltage-dependent anion-selective channel protein 2 (VDAC2)	I	229
Apoptosis-related proteins		
Bim/BOD (Bcl-2 interacting mediator of cell death/Bcl-2 related ovarian death gene)	I	121
DNA fragmentation factor 45 (DFF45)	D	230
p21	I	121
Procaspase-3	D	230
Procaspase-8	D	230
Procaspase-9	D	230
Receptor interacting protein (RIP)-like interacting CLARP kinase (RICK)	I	230
Zipper interacting protein kinase	I	121
Structural proteins		
Collagen I, alpha-1, polypeptide	Enriched in amyloid plaques	99
Coronin, actin binding protein	Enriched in amyloid plaques	99
Drebrin	D	231
Fibrinogen, gamma	Enriched in amyloid plaques	99
NF-L	D	232
Tau	Enriched in amyloid plaques	99
Vimentin	Enriched in amyloid plaques	99
Proteolysis		
Antitrypsin	Enriched in amyloid plaques	99
ATPase, Ca++ transporting plasma membrane protein 2	Enriched in amyloid plaques	99
ATPase, H+ transporting, lysosomal V0 subunit A	Enriched in amyloid plaques	99
ATPase, H+ transporting, lysosomal V1 subunit B	Enriched in amyloid plaques	99
ATPase, H+ transporting, lysosomal V1 subunit D	Enriched in amyloid plaques	99
ATPase, H+ transporting, lysosomal V1 subunit E	Enriched in amyloid plaques	99
Cathepsin D	Enriched in amyloid plaques	99
Cystatin B	Enriched in amyloid plaques	99
Cystatin C	Enriched in amyloid plaques	99
Ubiquitin-activating enzyme E1	Enriched in amyloid plaques	99
Vacuolar ATPase subunit H	Enriched in amyloid plaques	99
Glial associated proteins		
Glial fibrillary acidic protein (GFAP)	I	100
Membrane trafficking proteins		
Dynamin 1	Enriched in amyloid plaques	99
Dynein, heavy polypeptide 1	Enriched in amyloid plaques	99
Other		
Amyloid beta-peptide	Enriched in amyloid plaques	99
CSF proteins		
Alpha-1 antitrypsin	I	233
Alpha-1 beta glycoprotein	D	233
Apolipoprotein A1	D	233
Apolipoprotein E	D	233
Apolipoprotein J	D	233
Beta-2 microglobulin	I	234
Cell cycle progression 8 protein	D	233
Kininogen	D	233
Proapolipoprotein	D	234
Retinol-binding protein	D	233
Transthyretin	I	234