Journal of Alzheimer's Disease 33 (2013) S5–S13
DOI 10.3233/JAD-2012-129044
IOS Press



Review

# A Brief History of Alzheimer's Disease Gene Discovery

Rudolph E. Tanzi*
*Genetics and Aging Research Unit, Department of Neurology, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*

**Abstract**. The rich and colorful history of gene discovery in Alzheimer's disease (AD) over the past three decades is as complex and heterogeneous as the disease, itself. Twin and family studies indicate that genetic factors are estimated to play a role in at least 80% of AD cases. The inheritance of AD exhibits a dichotomous pattern. On one hand, rare mutations in *APP*, *PSEN1*, and *PSEN2* are fully penetrant for early-onset (<60 years) familial AD, which represents <5% of AD. On the other hand, common gene polymorphisms, such as the $\varepsilon$4 and $\varepsilon$2 variants of the APOE gene, influence susceptibility for common (>95%) late-onset AD. These four genes account for 30–50% of the inheritability of AD. Genome-wide association studies have recently led to the identification of additional highly confirmed AD candidate genes. Here, I review the past, present, and future of attempts to elucidate the complex and heterogeneous genetic underpinnings of AD along with some of the unique events that made these discoveries possible.

Keywords: Amyloid-β, amyloid-β protein precursor, APOE, CD33, chromosome 21, down syndrome, genome-wide association study, presenilin

## THE BEGINNING

In 1906, Dr. Alois Alzheimer's presented the brain autopsy results of Auguste Deter, a 55 year-old woman who had presenile dementia. Alzheimer described her as "a patient who was kept under close observation during institutionalization at the Frankfurt Hospital and whose central nervous system had been given to me by director Sioli for further examination". He then boldly proposed that her psychiatric problems were due to abnormal pathological lesions in her brain: miliary bodies (senile plaques) and fibrils inside of her neurons (neurofibrillary tangles). Alzheimer stated this was a "distinctive disease process" described by the correlation of clinical findings and pathological features. The esteemed audience was unimpressed by the idea that physical lesions could cause psychiatric symptoms. Nonetheless, Emil Kraeplin, Alzheimer's mentor, named presenile (onset <65 years) dementia "Alzheimer's disease" (AD). In the 1960 s and 1970 s, it became apparent that the same pathology littered the brains of patients who suffered from garden-variety, age-related dementia.

As a student in the neuroscience graduate program at Harvard Medical School in 1985, I had developed a keen interest in AD. In fact, I had already spent 1983–1984 testing for linkage of AD to some of the very first single nucleotide polymorphisms (SNPs; at that time, restriction fragment length polymorphisms; RFLPs) identified in the human genome. My mentor and doctoral advisor, Jim Gusella, together with Paul Watkins and I had identified these in the process of

*Correspondence to: Rudolph E. Tanzi, PhD, Genetics and Aging Research Unit, Department of Neurology, MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Harvard Medical School, 114 16th Street, Charlestown, MA 02129, USA. Tel.: +1 617 726 6845; Fax: +1 617 724 1949; E-mail: tanzi@helix.mgh.harvard.edu.

ISSN 1387-2877/13/$27.50 

localizing the Huntington's disease gene to chromosome 4. Huntington's disease was the first autosomal disease to be identified using SNPs, in this case RFLPs [1]. Soon afterward, my own research focused on whether the same could be accomplished for AD. So, I began testing our RFLPs for genetic linkage with early-onset familial AD (EO-FAD) (reviewed in [2]).

As a graduate student, I had also been reading with great interest about the heroic efforts of George Glenner and Colin Masters to purify the central core of amyloid-β (Aβ) in senile plaques and cerebrovascular deposits and determine the amino acid sequence of the Aβ protein. In their seminal study in 1984, Glenner and Wong [3] became the first to successfully purify and analyze cerebral Aβ deposits. They published the partial amino acid sequence of the peptide that they named the "β-protein". A year later, Colin Masters and colleagues confirmed and extended that "β-protein" sequence [4].

In his seminal paper [3], George Glenner also first proposed that AD is a cerebral amyloidosis and that cerebral Aβ drives all subsequent pathology in AD. This was the first reported formulation of what would later become known as the "amyloid hypothesis" of AD. George Glenner boldly proposed that the amyloid "β-protein" initiates the entire neuropathological process underlying AD. The same concept was reinterpreted and renamed the "amyloid cascade hypothesis" of AD in 1992 [5]. While this review is often referenced as the origin of the "amyloid hypothesis", from a historical perspective, it was actually George Glenner who had already put forward this hypothesis eight years earlier [3]. This hypothesis was still further refined in the years to come [6, 7]. Shortly after Glenner's report, Colin Masters and colleagues confirmed and extended the amino acid sequence of the Aβ protein [4], which he and Konrad Beyreuther referred to as "A4". Later, the combination of the names A4 and amyloid-β protein were combined and abbreviated to "Aβ". Glenner and Wong [3] also predicted that the gene responsible for making Aβ in senile plaques and cerebrovascular deposits, would be on chromosome 21 and that it would carry mutations causing AD. Their prediction was based on the observation that middle-aged and older Down syndrome (trisomy 21) patients exhibit AD neuropathology.

In Jim Gusella's laboratory, we already had several EO-FAD pedigrees that I had begun testing for genetic linkage to some of the original human SNPs, isolated in our laboratory. Also, beginning around 1983, we began isolating chromosome-specific SNPs, focusing on chromosome 21 [8]. We used these SNPs as genetic markers to build a complete "end-to-end" genetic linkage map of chromosome 21 [9]. We then used our linkage map of chromosome 21 to assess genotype-phenotype correlations in Down syndrome and recombination patterns as parents age. For example, we tested whether recombination was altered in older mothers perhaps leading to mitotic nondisjunction and trisomy 21. We found that as mothers got older, recombination breakpoints on chromosome 21 moved from the telomeres to the middle of chromosome 21 while recombination frequencies were also reduced [10]. But, our main focus was to use the chromosome 21 markers and map to test for genetic linkage to EO-FAD.

On the heels of Glenner's bold prediction that the gene responsible for the β-protein would be on chromosome 21 and carry mutations causing AD, I decided to focus my doctoral studies in the Neuroscience Program at Harvard Medical School. I set out to test this hypothesis by attempting to isolate the β-protein gene and test it for genetic linkage to EO-FAD. We hoped that by identifying the gene for Aβ we could discover some of the first molecular clues to the cause of AD. Up to this point, guesses about what causes AD ranged from viral infections to leakage of unknown pathogens into the brain from a compromised blood brain barrier in the elderly. So, in 1984, I embarked on a project to isolate Aβ gene. What I did not know was that I had also entered into an "undeclared" race with laboratories around the world that had likewise resonated with Glenner's prediction that Aβ drove AD and that the Aβ gene would be an AD gene [3]. To isolate the gene responsible for producing the Aβ protein, we used the amino acid sequences for Aβ reported by the laboratories of Glenner and Masters [3, 4]. This was, in essence, the first AD candidate gene search.

## THE EARLY-ONSET ALZHEIMER'S DISEASE GENES

In 1983, in Jim Gusella's laboratory, I had begun testing a large Canadian EO-FAD family for linkage to the markers (RFLPs) comprising our full genetic linkage map of chromosome 21. Our AD linkage results for all RFLPs spanning our chromosome 21 genetic linkage map were entirely negative for the Canadian EO-FAD family. Jim Gusella's laboratory then collected a second FAD family of Italian origin with the help of Robert Feldman (Boston University) and Jean-Francois Foncin (La Salpetriere Hospital, Paris). In 1985, I tested all of our chromosome 21 markers in

the Italian EO-FAD family. But once again, all of the signals were negative; we found no evidence of genetic linkage to AD on chromosome 21 after testing the first two multi-generational EO-FAD families. A short time later, Peter St. George-Hyslop had joined Jim Gusella's laboratory as a postdoctoral fellow. As I headed off to David Kurnit's laboratory at Boston Children's Hospital (for my doctoral thesis at Harvard) to try to isolate the Aβ gene, Peter St. George-Hyslop assumed responsibility for the FAD-chromosome 21 linkage study. By this time, Jim Gusella's laboratory had added two additional EO-FAD kindreds, one from Germany and one from Russia bringing the total to four and Peter St. George-Hyslop continued testing our chromosome 21 markers in those families.

To isolate the first AD candidate gene, that responsible for the production of Aβ, George Glenner's β-protein [3] and Colin Masters' A4 [4], we employed a "reverse genetics" strategy. As a graduate student, I was doing a rotation in the laboratory of the late Dr. David Kurnit, a renowned Down's syndrome geneticist at Boston Children's Hospital. He had me collaborate with his postdoctoral fellow, Rachael Neve, who was making various high quality fetal human cDNA libraries. My close collaborator on the chromosome 21 mapping study, Paul Watkins, then helped design and synthesized our two oligonucleotides corresponding to the β-protein/A4 amino acid sequences published by the groups of Glenner and Masters [3, 4]. We used two non-overlapping oligonucleotides corresponding to the β-protein/A4 amino acid sequence as probes: one was a 21-mer corresponding to amino acids 1–7, and the second was a 48-mer corresponding to amino acids 9–24.

To clone the Aβ gene, Paul and I devised a strategy we called the "genomic window" approach. The strategy utilized Southern blots with six different human DNAs cleaved with 15 different restriction enzymes, run in separate lanes. These Southern blots were similar to those we had originally used to identify the first RFLPs, which were used to localize the Huntington's disease gene [1]. We first determined which restriction fragments the two non-overlapping oligonucleotides detected in common on the Southern blots containing human DNA cleaved with various restriction endonucleases. We then argued that the most promising candidate Aβ gene clones detected by the two oligonucleotides would detect most of these same common restriction fragments on the Southern blots.

We screened human fetal brain, eye, and liver cDNA libraries with the two non-overlapping oligonucleotides and picked cDNA clones that hybridized independently to both oligonucleotide probes. Next, we asked which cDNA clones detected highly similar sets of human restriction fragments as the two non-overlapping oligonucleotides. Then, in a true leap of faith, betting that Glenner's prediction [3] was correct, we also tested whether any of the Aβ cDNA clones meeting the above criteria also hybridized to human-rodent somatic cell hybrid cell lines, which contained only human chromosome 21 as their human genetic material. Using this method, we found cDNA clones that met all these criteria, including one that was considered to be our top candidate Aβ cDNA clones.

That Fall, as a graduate student, I attended the 1986 Society for Neuroscience meeting with my new β-protein/A4 data and Southern blots in my briefcase. At one of the AD sessions, we had learned that the chairperson was permitting an unscheduled speaker to make a slide presentation. The speaker was Dmitry Goldgaber, who we learned had also been attempting to find the Aβ gene at the NIH, working under Nobel laureate, Carlton Gajdusek. Dmitry Goldgaber presented evidence that he had isolated a cDNA encoding the amino acids in the published Aβ protein sequence. After the session, I met Dmitry Goldgaber and informed him that I was a graduate student who had also been screening for the β-protein/A4 gene, and that our top cDNA contained the same sized ~1.1 kb EcoRI fragment as the cDNA clone he had just presented. Like me, Dmitry Goldgaber was eager to find out whether the gene responsible for Aβ was linked to EO-FAD. So, he invited me to his laboratory at the NIH. There, I met with his collaborator, geneticist, Wesley McBride. When Wesley McBride and I compared the human DNA restriction fragments detected by our respective Aβ cDNAs on Southern blots, we concluded that our cDNA clones were identical, albeit from different cDNA libraries. Nonetheless, Dmitry Goldgaber requested that I take his clone back to Boston, just in case. A few months later, when we reported the identification and characterization of our respective β-protein/A4 (Aβ) cDNA clones in back-to-back papers in Science, we both acknowledged the pre-publication comparison in our reports [11, 12].

In our β-protein/A4/Aβ gene (later renamed the *APP* gene) cloning paper [12], we showed that our cDNA was derived from a single copy gene on chromosome 21 based on hybridization to a whole genome somatic cell hybrid panel. We also determined that the *APP* gene corresponded to a 3.2 kb message ubiquitously expressed in the body with highest levels in the brain, heart, kidney, spleen, and pancreas. In the brain, we observed the highest mRNA levels in A40,

A44, A20/21, A10, and cerebellar cortex [12]. We also observed that an extra copy of the gene led to excessive amounts of mRNA in Down syndrome patients [12]. This nicely supported Glenner's original prediction that Down syndrome patients accumulate excessive amounts of Aβ protein owing to an extra copy of the gene on chromosome 21.

We also used our chromosome 21 genetic linkage map [9] to localize the *APP* gene near the chromosome 21 RFLP marker *D21S1* [12]. This was very interesting because back in 1983–1984, I had not been able to detect any significant genetic linkage of EO-FAD to the markers on my chromosome 21 map in the first two (of four) EO-FAD families being studied in Jim Gusella's laboratory at Massachusetts General Hospital. However, Peter St. George-Hyslop had continued the linkage studies in four total EO-FAD families together with Jonathan Haines; in the Summer 1986, they claimed to find genetic linkage of FAD to the region of chromosome 21 around the same polymorphic marker, *D21S1*, which we had mapped close by *APP* [12]. Most of their evidence for linkage of EO-FAD to the *D21S1-APP* region of chromosome 21 came from the Italian EO-FAD family, which I had tested two years earlier with negative results. The difference was that they carried out multi-locus genetic linkage analysis including additional chromosome 21 markers, while I only carried out single locus analyses on fewer markers back in 1983–1984.

Our paper [12] describing the cloning of the *APP* gene, its mapping to chromosome 21, its expression profiles in body and brain, and overexpression in Down syndrome, was published in February 1987 in *Science* back-to-back with St. George-Hylop's paper claiming linkage of EO-FAD to chromosome 21 in the region near *APP* [13], and also back-to-back with Dmitry Goldgaber's *APP* cloning paper [11]. Another study published in Nature the same week reported a full-length *APP* cDNA [14]. Later, in 1988, we and others also reported an alternatively spliced form of APP containing a Kunitz protease inhibitor (KPI) domain [15].

By early 1987, I had also completed the experiments aimed at testing whether the *APP* gene was linked to FAD in the four Massachusetts General Hospital EO-FAD pedigrees by analyzing the segregation of *APP* gene RFLPs in all four families. The genetic linkage results were all strikingly negative. *APP* was clearly not the genetic culprit in these four FAD kindreds [16]. This finding meant that even if there were a gene on chromosome 21 responsible for FAD in these four pedigrees, as purported in the St. George-Hyslop et al. [13], it was *not* APP. (Later, these same four FAD pedigrees were shown to actually be linked to chromosome 14 and, ultimately, to contain mutations in the presenilin 1 (*PSEN1*) gene [17]). Christine Van Broeckhoven, John Hardy, and colleagues also found lack of linkage of EO-FAD to APP in their families [18]. In both of our papers [16, 18], *APP* was ruled from linkage in the specific EO-FAD families tested, but was not ruled out as a candidate gene for all of AD.

Ironically, the spurious linkage of these EO-FAD families to chromosome 21 [13] ended up serving an important role. The publication motivated other groups, most notably that of John Hardy, to test their own FAD families for linkage to chromosome 21. Inspired by the St. George-Hyslop et al. study [13], Hardy soon identified EO-FAD families that actually *were* linked to chromosome 21. Later, in 1990, the first pathogenic mutation in *APP* was reported by Levy et al. in 1990 [19]. By sequencing of exons 16 and 17 of *APP*, encoding the Aβ portion, they found a mutation in *APP* responsible for Dutch hereditary cerebral hemorrhage with amyloidosis, sometimes referred to as "vascular AD". Following up on the APP mutation discovered by Levy et al. [19], Hardy and colleagues then re-sequenced the same two exons (16 and 17) of *APP* in the EO-FAD families that they had previously linked to chromosome 21 (based on the report by St. George-Hyslop et al. [13]), and found the EO-FAD *APP* mutation known as the "London mutation" (V717I) [20].

Meanwhile, our own attempts to find EO-FAD mutations in *APP* were futile. None of the 50 or so EO-FAD families for which we re-sequenced exons 16 and 17 of *APP* between 1987 and 1988 revealed mutation [21]. Soon after the four EO-FAD families reported earlier to be linked to chromosome 21 [13] were found to actually harbor mutations in the *PSEN1* gene [17], we collaborated with Jerry Schellenberg to identify a homolog of *PSEN1* called presenilin (*PSEN2*) [22]. It was mapped to chromosome 1 and in June 1995, we showed that it harbored the N141I mutation causing AD Volga-German EOFAD families [22]. This finding subsequently confirmed [23].

To date, well over 200 EO-FAD mutations (plus duplications) have been reported for *APP, PSEN1*, and *PSEN2* (http://www.molgen.ua.ac.be/ADMutations). While the EO-FAD mutations in *APP, PSEN1*, and *PSEN2* and duplication of *APP* are all considered fully penetrant, recently we reported the first case of non-penetrant duplication of *APP* [24]. Most of the EO-FAD mutations are in PSEN1 and the majority of them, in all three of the genes, lead to an increase in the ratio of $A\beta_{42} : A\beta_{40}$ [7, 25]. Based on this knowledge,

γ-secretase modulators aimed at reversing this ratio are being developed in my laboratory and that of Steve Wagner at UCSD. These APP-selective γ-secretase modulators carry great promise for treating and preventing AD [26]. And, their development was made possible by the identification and characterization of the three EO-FAD genes.

While the chromosome 21 genetic linkage map was primarily being used to investigate Down syndrome and EO-FAD, it was also used to help establish linkage of familial amyotrophic lateral sclerosis (ALS) to chromosome 21 [27]. This led to our collaboration with the laboratory of Bob Brown, who discovered the first familial ALS mutations in the gene for superoxide dismutase 1 (*SOD1*). This discovery was facilitated by an international collaboration aimed at physically mapping chromosome 21 using yeast artificial chromosomes (YACs). The effort was being coordinated out of my laboratory in the early- to mid-1990 s and was aimed at producing a combined physical and genetic map of chromosome 21. Since this was all before email, we distributed the updated physical mapping information of chromosome 21 in various YACs by fax in a weekly report called "The Fax on the YACs". When the Brown laboratory discovered strong genetic linkage with no recombination events for familial ALS and the chromosome 21 RFLP, DB1, Sandy Gaston, a postdoctoral fellow in my laboratory, mapped DB1 to a YAC that contained SOD1 as its only gene. Subsequently, SOD1 was sequenced in the DB1-linked familial ALS families leading to the first ALS mutations in this gene in 1993 [28].

In 1993, we also reported that one of the non-*APP* cDNA's originally pulled out with the oligonucleotides designed against the Aβ protein amino acid sequence in 1986 was the gene for Wilson's disease, a neurodegenerative disorder characterized by copper toxicity [29]. Based on this finding, I had my postdoctoral fellow, Ashley Bush, test for the ability of the Aβ protein to bind various metals, including copper and zinc. We then reported that copper and zinc aggressively drove the aggregation of the Aβ protein [30]. Based on that finding, in 1997, Ashley and I, together with Geoffrey Kempler, co-founded a company in my laboratory called Prana Biotechnology. The goal of Prana was to develop novel therapeutics for AD drugs based on their ability to prevent the interaction of copper and zinc with the Aβ protein. This was based on what we called "The Metal Hypothesis of AD" [31]. In 2001, Prana Biotechnology, for which I serve as chief scientific advisor, moved to Australia, and is now developing a highly promising metal chaperone drug called PBT2 as a therapeutic for AD. The drug dramatically ameliorated AD pathology in transgenic AD mice and successfully improved cognition in a Phase IIA clinical trial [31]. PBT2 is being further developed for the treatment of both AD and Huntington's disease in ongoing clinical trials.

## THE LATE-ONSET ALZHEIMER'S DISEASE GENES

Late-onset Alzheimer's (LOAD) is the most common form of the disease with onset age >65 years. LOAD is characterized by a genetically complex and heterogeneous pattern of inheritance. It is generally assumed that for most cases of LOAD, genetic factors work together with lifestyle and environmental factors to determine one's risk for AD. The best-established LOAD risk factor is the ε4 variant of the apolipoprotein E gene (*APOE*) on chromosome 19 [32]. The identification of *APOE* as an AD gene came from the combination of genetic linkage of LOAD to chromosome 19 near *APOE* and the finding that ApoE was bound to Aβ in cerebrospinal fluid [32]. One copy of the ε4-allele of *APOE* increases AD risk by ∼4-fold and two copies, greater than ten-fold; the ε2-allele of *APOE* is protective for AD [33]. Functionally, APOE maintains lipid metabolism and transport, but in AD pathogenesis is believed to play a role in the clearance of cerebral Aβ.

After the report of association of LOAD with *APOE*, many hundreds of genes were tested for association with AD leading to thousands of studies reporting positive associations, lack of association, replications, and refutations. To keep track of these studies, Lars Bertram and I started the online database, AlzGene.org (http://www.alzgene.org) [34, 35]. AlzGene provides meta-analysis results for all AD candidate genes that have been tested in at least four independent case-control samples. Currently, AlzGene.org meta-analyses reveal over three-dozen loci that show nominally significant association with risk for AD and ten with nominal $p$-values $<10^{-5}$. The ten loci with the strongest evidence for association with AD are *APOE* and nine more that derived from genome-wide association studies (GWAS) [34, 35].

In 2008, my laboratory reported the first genes to exhibit genome-wide significant association with AD based on "family-based" GWAS [36]. At that time, we reported three novel AD genes candidates including ataxin 1 (*ATXN1*), siglec 3 (*CD33*), and a locus on chromosome 14 (GWA_14q31.2). *ATXN1* can contain

an expanded poly-glutamine repeat causing spinocerebellar ataxia type 1. We have since shown that *ATXN1* affects Aβ levels by modulating β-secretase levels and cleavage of APP [37]. *CD33* is a sialic acid-binding, immunoglobulin-like lectin that regulates the innate immune system [38]. This is interesting given our recent discovery that Aβ possesses the properties of an antimicrobial peptide, suggesting that it may play a role in the brain's innate immune system [39].

In 2009, a case-control-based GWAS reported association of AD with *CLU* (clusterin; apolipoprotein J), *CR1* (complement component (3b/4b) receptor 1), and *PICALM* (phosphatidylinositol binding clathrin assembly protein) [40, 41]. In 2010, *BIN1* (bridging integrator 1) was reported to exhibit genome-wide significance for association with LOAD [42]. And in 2011, two more case-control-based GWAS reported four more AD genes: *CD2AP, MS4A6A/MS4A4E, EPHA1,* and *ABCA* [43, 44]. These same GWAS further supported our genome-wide significant association of *CD33* with AD, which we had reported three years earlier [36]. The 2011 GWAS studies identified an additional SNP (∼1400 bp away from ours) in *CD33* as a risk factor AD. Overall, these confirmed AD gene candidates coming out of GWAS roughly fall into four categories: Aβ metabolism, lipid metabolism, innate immunity, and cell signaling.

More recently, our laboratory has found two rare, highly penetrant mutations for in *ADAM10,* which encodes the major α-secretase in the brain. α-secretase cleaves APP within the Aβ to preclude Aβ [45]. We reported two rare (7 of 1000 LOAD families) *ADAM10* LOAD mutations, Q170H and R181G, both of which are located in the prodomain region. We showed that both mutations dramatically impair the ability of ADAM10 to cleave APP at the α-secretase site of APP *in vitro* [45] and *in vivo* (unpublished observations). This study [45] is the first (and to date, only) to report rare highly penetrant mutations for LOAD. The existence of these two mutations in *ADAM10* underscores the importance of the future employment of whole genome or whole exome sequencing to find rare variants causing LOAD, in addition to common variants.

## CONCLUSIONS

Most would agree that prior to the identification of the AD genes our understanding of the causes of AD was minimal. Thanks to the efforts of dozens of laboratories over the past three decades, genetics has taught us volumes about the etiology and pathogenesis of AD along with clues for treatment and prevention [7, 46]. The 1980 s and 1990 s can be considered a golden age for AD genetics based on the oft-times circuitous elucidation of the established AD genes, *APP, PSEN1, PSEN2,* and *APOE*. While, the field of AD genetics then experienced a relative lull in genetic discovery between 1995 and 2005, GWAS, which screens the entire human genome for novel AD loci [46, 47] has ushered in a new era of AD genetics over the past five years. Now, with affordable whole genome sequencing close on the heels of GWAS, a new golden era of AD gene discovery will hopefully soon be upon us, and deliver new clues regarding the causes of AD as well as the most effective means for treatment and prevention.

## DISCLOSURE STATEMENT

The author's disclosure is available online (http://www.j-alz.com/disclosures/view.php?id=1489).

## REFERENCES

[1] Gusella JF, Wexler NS, Conneally PM, Naylor SL, Anderson MA, Tanzi RE, Watkins PC, Ottina K, Wallace MR, Sakaguchi AY, Young AB, Shoulson I, Bonilla E, Martin JB (1983) A polymorphic DNA marker genetically linked to Huntington's disease. *Nature* **306**, 234-238.

[2] Tanzi RE, Parson AB (2000) *Decoding darkness: The search for the genetic causes of Alzheimer's disease*. Basic Books, New York, p. 304.

[3] Glenner GG and Wong CW (1984) Alzheimer's disease and Down's syndrome: Sharing of a unique cerebrovascular amyloid fibril protein. *Biochem Biophys Res Commun* **122**, 1131-1135.

[4] Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci U S A* **82**, 4245-4249.

[5] Hardy JA, Higgins GA (1992) Alzheimer's disease: The amyloid cascade hypothesis. *Science* **256**, 184-185.

[6] Hardy. J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* **297**, 353-356.

[7] Tanzi RE, Bertram L (2005) Twenty years of the Alzheimer's disease amyloid hypothesis: A genetic perspective. *Cell* **4**, 545-555.

[8] Watkins PC, Tanzi RE, Gibbons KR, Tricoli JV, Landes G, Eddy R, Shows TB, Gusella JF (1985) Isolation of polymorphic DNA segments from human chromosome 21. *Nucleic Acids Res* **13**, 6075-6089.

[9] Tanzi RE, Haines JL, Watkins PC, Stewart GD, Hallewell R, Wong C, Wexler NS, Conneally PM, Gusella JF (1988) Genetic linkage map of human chromosome 21. *Genomics* **3**, 129-136.

[10] Tanzi RE, Watkins PC, Stewart GD, Wexler NS, Gusella JF, Haines JL (1992) A genetic linkage map of human

chromosome 21q: Patterns of recombination according to age and sex. *Am J Hum Genet* **50**, 551-558.

[11] Goldgaber D, Lerman MI, McBride OW, Saffiotti U, Gajdusek DC (1987) Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science* **235**, 877-880.

[12] Tanzi RE, Gusella JF, Watkins PC, Bruns GA, St George-Hyslop P, Van Keuren ML, Patterson D, Pagan S, Kurnit DM, Neve RL (1987) Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* **235**, 880-884.

[13] St. George-Hyslop PH, Tanzi RE, Polinsky RJ, Haines JL, Nee L, Watkins PC, Myers R, Feldman R, Pollen D, Drachman D, Growdon J, Bruni A, Foncin J-F, Frommelt P, Amaducci L, Sorbi S, Piacentini S, Stewart GD, Hobbs WJ, Conneally PM, Gusella JF (1987) The genetic defect causing familial Alzheimer's disease maps on chromosome 21. *Science* **235**, 885-890.

[14] Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Muller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* **325**, 733-736.

[15] Tanzi RE, McClatchey AI, Lamperti ED, V-Komaroff L, Gusella JF, Neve R (1988) Protease inhibitor domain encoded by an amyloid protein precursor mRNA associated with Alzheimer's disease. *Nature* **331**, 528-530.

[16] Tanzi RE, St. George-Hyslop PH, Haines JL, Polinsky RJ, Nee L, Foncin J-F, Neve RL, McClatchey AI, Conneally PM, Gusella JF (1987) The genetic defect in familial Alzheimer's disease is not tightly linked to the amyloid beta protein gene. *Nature* **329**, 156-157.

[17] Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HA, Haines JL, Perkicak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St George-Hyslop PH (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375**, 754-760.

[18] Van Broeckhoven C, Genthe AM, Vandenberghe A, Horsthemke B, Backhovens H, Raeymaekers P, Van Hul W, Wehnert A, Gheuens J, Cras P, Bruyland M, Martin J, Salbaum M, Multhaup G, Masters C, Beyreuther K, Gurling HMD, Mullan MJ, Haolland A, Barton A, Irving N, Williamson R, Richards SJ, Hardy JA (1987) Failure of familial Alzheimer's disease to segregate with the A4-amyloid gene in several European families. *Nature* **329**, 153-155.

[19] Levy E, Carman MD, Fernandez-Madrid IJ, Power MD, Lieberburg I, van Duinen SG, Bots GT, Luyendijk W, Frangione B (1990) Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch type. *Science* **248**, 1124-1126.

[20] Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349**, 704-706.

[21] Tanzi RE, Vaula G, Romano DM, Mortilla M, Huang TL, Tupler RG, Wasco W, Hyman BT, Haines JL, Jenkins BJ, Kalaitsidaki M, Warren AC, McInnis MG, Antonarakis SE, Karlinsky H, Percy ME, Connor L, Growdon J, Crapper-Mclachlan DR, Gusella JF, St. George-Hyslop PH (1992) Assessment of β-amyloid protein precursor gene mutations in a large set of familial and sporadic Alzheimer disease cases. *Am J Hum Genet* **51**, 273-282.

[22] Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH, Yu CE, Jondro PD, Schmidt SD, Wang K, Crowley AC, Fu Y-H, Guenette SY, Galas D, Nemens E, Wijsman EM, Bird TD, Schellenberg GD, Tanzi RE (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269**, 973-977.

[23] Rogaev EI, Sherrington R, Rogaeva EA, Levesque G, Ikeda M, Liang Y, Chi H, Lin C, Holman K, Tsuda T (1995) 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* **376**, 775-778.

[24] Hooli BV, Mohapatra G, Mattheisen M, Parrado AR, Roehr JT, Shen Y, Gusella JF, Moir R, Saunders AJ, Lange C, Tanzi RE, Bertram L (2012) Role of common and rare APP DNA sequence variants in Alzheimer disease. *Neurology* **78**, 1250-1257.

[25] Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W et al. (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* **2**, 864-870.

[26] Kounnas MZ, Danks AM, Cheng S, Tyree C, Ackerman E, Zhang X, Ahn K, Nguyen P, Comer P, Mao L, Yu C, Pleynet D, Digregorio PJ, Velicelebi G, Stauderman KA, Comer WT, Mobley WC, Li Y-M, Sisodia SS, Tanzi RE, Wagner SL (2010) Modulation of γ-secretase reduces β-amyloid deposition in a transgenic mouse model of Alzheimer's disease. *Neuron* **67**, 769-780.

[27] Siddique T, Figlewicz M, Perikak-Vance M, Haines J, Roos RP, Rouleau G, Williams D, Watkins PC, Jeffers A, Sapp P, Hung W, Bebout J, Noore FR, Nicholson G, Reed R, McKenna-Yasek D, Deng G, Beard C, Brooks BR, Festoff B, Antel J, Tandan R, Munsat TL, Mulder DW, Laing NG, Halperin J, Norris FH, Van den Bergh R, Swerts L, Tanzi R, Jubelt B, Matthews KM, Bosch EP, Horvitz R, Gusella JF, Brown RH, Roses AD (1991) Assignment of a gene causing familial amyotrophic lateral sclerosis to chromosome 21 and evidence for genetic heterogeneity. *N Eng J Med* **324**, 1381-1384.

[28] Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hontati A, Donaldson D, Goto J, O'Regan JP, Deng H-X, Rahman Z, Krizus A, McKenna-Yasek D, Tanzi RE, Halperin JJ, Herzfeldt B, Van den Berg R, Hung W-Y, Bird T, Deng G, Mulder DW, Smyth C, Laing NG, Soriano E, Pericak-Vance M, Haines J, Rouleau GA, Horvitz HR, Brown RH (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**, 59-62.

[29] Tanzi RE, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Ross B, Romano DM, Brzustowicz LM, Devoto M, Peppercorn J, Bush AI, Sternlieb I, Pirastu M, Gusella JF, Evgrafov O, Penchaszadeh GK, Honig B, Edelman IS, Soares MB, Scheinberg IH, Gilliam TC (1993) Identification of the Wilson's disease gene: A copper transporting ATPase with homology to the Menke's disease gene. *Nat Genet* **5**, 344-350.

[30] Bush AI, Pettingell W, Multhaup G, Paradis MD, Vonsattel J-P, Gusella JF, Beyreuther K, Masters CL, Tanzi RE (1994) Rapid induction of Alzheimer Aβ amyloid formation by zinc. *Science* **265**, 1464-1467.

[31] Bush AI, Tanzi RE (2008) Therapeutics for Alzheimer's disease based on the metal hypothesis. *Neurotherapeutics* **5**, 421-432.

[32] Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993) Apolipoprotein E: High-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A* **90**, 1977-1981.

[33] Corder EH, Saunders AM, Risch NJ, Strittmatter WJ, Schmechel DE, Gaskell PC Jr, Rimmler JB, Locke PA, Conneally PM, Schmader KE et al. (1994) Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nat Genet* **7**, 180-184.

[34] Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE (2007) Systematic meta-analyses of Alzheimer disease genetic association studies: The AlzGene database. *Nat Genet* **39**, 17-23.

[35] Bertram L, Tanzi RE (2008) Thirty years of Alzheimer's disease genetics: Systematic meta-analyses herald a new era. *Nat Rev Neurosci* **9**, 768-778.

[36] Bertram L, Lange C, Mullin K, Parkinson M, Hsiao M, Hogan MF, Schjeide BMM, Hooli B, Divito J, Ionita I, Jiang H, Laird N, Moscarillo T, Ohlsen KL, Elliott K, Wang X, Hu-Lince D, Ryder M, Murphy A, Wagner SL, Blacker D, Becker KD, Tanzi RE (2008) Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. *Am J Hum Genet* **5**, 623-632.

[37] Zhang C, Browne A, Child D, Divito JR, Stevenson JA, Tanzi RE (2010) Loss of function of ATXN1 increases amyloid beta-protein levels by potentiating beta-secretase processing of beta-amyloid precursor protein. *J Biol Chem* **285**, 8515-8526.

[38] Crocker PR, Paulson JC, Varki A (2007) Siglecs and their roles in the immune system. *Nat Rev Immunol* **7**, 255-266.

[39] Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, Burton MA, Goldstein LE, Duong S, Tanzi RE, Moir RD (2010) The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. *PLoS ONE* **5**, e9505.

[40] Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Williams A, Jones N, Thomas C, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Hardy J, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schürmann B, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H, Hüll M, Rujescu D, Goate AM, Kauwe JSK, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel K, Klopp N, Wichmann H, Carrasquillo MM, Pankratz VS, Younkin SG, Holmans PA, O'Donovan M, Owen MJ, Williams J (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet* **41**, 1088-1093.

[41] Lambert J, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, Combarros O, Zelenika D, Bullido MJ, Tavernier B, Letenneur L, Bettens K, Berr C, Pasquier F, Fiévet N, Barberger-Gateau P, Engelborghs S, De Deyn P, Mateo I, Franck A, Helisalmi S, Porcellini E, Hanon O, de Pancorbo MM, Lendon C, Dufouil C, Jaillard C, Leveillard T, Alvarez V, Bosco P, Mancuso M, Panza F, Nacmias B, Bossù P, Piccardi P, Annoni G, Seripa D, Galimberti D, Hannequin D, Licastro F, Soininen H, Ritchie K, Blanché H, Dartigues J, Tzourio C, Gut I, Van Broeckhoven C, Alpérovitch A, Lathrop M, Amouyel P (2009) Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet* **41**, 1094-1099.

[42] Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, Bis JC, Smith AV, Carassquillo MM, Lambert JC, Harold D, Schrijvers EMC, Ramirez-Lorca R, Debette S, Longstreth WT, Janssens ACJW, Pankratz VS, Dartigues JF, Hollingworth P, Aspelund T, Hernandez I, Beiser A, Kuller LH, Koudstaal PJ, Dickson DW, Tzourio C, Abraham R, Antunez C, Du Y, Rotter JI, Aulchenko YS, Harris TB, Petersen RC, Berr C, Owen MJ, Lopez-Arrieta J, Varadarajan BN, Becker JT, Rivadeneira F, Nalls MA, Graff-Radford NR, Campion D, Auerbach S, Rice K, Hofman A, Jonsson PV, Schmidt H, Lathrop M, Mosley TH, Au R, Psaty BM, Uitterlinden AG, Farrer LA, Lumley T, Ruiz A, Williams J, Amouyel P, Younkin SG, Wolf PA, Launer LJ, Lopez OL, van Duijn CM, Breteler MMB (2010) Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA* **303**, 1832-1840.

[43] Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK, Larson EB, Bird TD, Boeve BF, Graff-Radford NR, De Jager PL, Evans D, Schneider JA, Carrasquillo MM, Ertekin-Taner N, Younkin SG, Cruchaga C, Kauwe JS, Nowotny P, Kramer P, Hardy J, Huentelman MJ, Myers AJ, Barmada MM, Demirci FY, Baldwin CT, Green RC, Rogaeva E, St George-Hyslop P, Arnold SE, Barber R, Beach T, Bigio EH, Bowen JD, Boxer A, Burke JR, Cairns NJ, Carlson CS, Carney RM, Carroll SL, Chui HC, Clark DG, Corneveaux J, Cotman CW, Cummings JL, DeCarli C, DeKosky ST, Diaz-Arrastia R, Dick M, Dickson DW, Ellis WG, Faber KM, Fallon KB, Farlow MR, Ferris S, Frosch MP, Galasko DR, Ganguli M, Gearing M, Geschwind DH, Ghetti B, Gilbert JR, Gilman S, Giordani B, Glass JD, Growdon JH, Hamilton RL, Harrell LE, Head E, Honig LS, Hulette CM, Hyman BT, Jicha GA, Jin LW, Johnson N, Karlawish J, Karydas A, Kaye JA, Kim R, Koo EH, Kowall NW, Lah JJ, Levey AI, Lieberman AP, Lopez OL, Mack WJ, Marson DC, Martiniuk F, Mash DC, Masliah E, McCormick WC, McCurry SM, McDavid AN, McKee AC, Mesulam M, Miller BL, Miller CA, Miller JW, Parisi JE, Perl DP, Peskind E, Petersen RC, Poon WW, Quinn JF, Rajbhandary RA, Raskind M, Reisberg B, Ringman JM, Roberson ED, Rosenberg RN, Sano M, Schneider LS, Seeley W, Shelanski ML, Slifer MA, Smith CD, Sonnen JA, Spina S, Stern RA, Tanzi RE, Trojanowski JQ, Troncoso JC, Van Deerlin VM, Vinters HV, Vonsattel JP, Weintraub S, Welsh-Bohmer KA, Williamson J, Woltjer RL, Cantwell LB, Dombroski BA, Beekly D, Lunetta KL, Martin ER, Kamboh MI, Saykin AJ, Reiman EM, Bennett DA, Morris JC, Montine TJ, Goate AM, Blacker D, Tsuang DW, Hakonarson H, Kukull WA, Foroud TM, Haines JL, Mayeux R, Pericak-Vance MA, Farrer LA, Schellenberg GD (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet* **43**, 436-441.

[44] Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Jones N, Stretton A, Thomas C, Richards A, Ivanov D, Widdowson C, Chapman J, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Beaumont H, Warden D, Wilcock G, Love S, Kehoe PG, Hooper NM, Vardy ER, Hardy J, Mead S, Fox NC, Rossor M,

Collinge J, Maier W, Jessen F, Rüther E, Schürmann B, Heun R, Kölsch H, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H, Gallacher J, Hüll M, Rujescu D, Giegling I, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel KH, Klopp N, Wichmann HE, Pankratz VS, Sando SB, Aasly JO, Barcikowska M, Wszolek ZK, Dickson DW, Graff-Radford NR, Petersen RC, Alzheimer's Disease Neuroimaging Initiative, van Duijn CM, Breteler MM, Ikram MA, DeStefano AL, Fitzpatrick AL, Lopez O, Launer LJ, Seshadri S, consortium CHARGE, Berr C, Campion D, Epelbaum J, Dartigues JF, Tzourio C, Alpérovitch A, Lathrop M, EADI1 consortium, Feulner TM, Friedrich P, Riehle C, Krawczak M, Schreiber S, Mayhaus M, Nicolhaus S, Wagenpfeil S, Steinberg S, Stefansson H, Stefansson K, Snaedal J, Björnsson S, Jonsson PV, Chouraki V, Genier-Boley B, Hiltunen M, Soininen H, Combarros O, Zelenika D, Delepine M, Bullido MJ, Pasquier F, Mateo I, Frank-Garcia A, Porcellini E, Hanon O, Coto E, Alvarez V, Bosco P, Siciliano G, Mancuso M, Panza F, Solfrizzi V, Nacmias B, Sorbi S, Bossù P, Piccardi P, Arosio B, Annoni G, Seripa D, Pilotto A, Scarpini E, Galimberti D, Brice A, Hannequin D, Licastro F, Jones L, Holmans PA, Jonsson T, Riemenschneider M, Morgan K, Younkin SG, Owen MJ, O'Donovan M, Amouyel P, Williams J (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet* **43**, 429-435.

[45] Kim M, Suh J, Romano D, Truong MH, Mullin K, Hooli B, Norton D, Tesco G, Elliott K, Wagner SL, Moir RD, Becker KD, Tanzi RE (2009) Potential late-onset Alzheimer's disease-associated mutations in the ADAM10 gene attenuate a-secretase activity. *Hum Mol Genet* **18**, 3987-3996.

[46] Bertram L, Lill CM, Tanzi RE (2010) The genetics of Alzheimer disease: Back to the future. *Neuron* **68**, 270-281.

[47] Bertram L, Tanzi RE (2009) Genome-wide association studies in Alzheimer's disease. *Hum Mol Genet* **18**, R137-R145.