Review

Khue Vu Nguyen*

The human β-amyloid precursor protein: biomolecular and epigenetic aspects

Abstract: Beta-amyloid precursor protein (APP) is a membrane-spanning protein with a large extracellular domain and a much smaller intracellular domain. APP plays a central role in Alzheimer's disease (AD) pathogenesis: APP processing generates β-amyloid (Aβ) peptides, which are deposited as amyloid plaques in the brains of AD individuals; point mutations and duplications of *APP* are causal for a subset of early-onset familial AD (FAD) (onset age <65 years old). However, these mutations in FAD represent a very small percentage of cases (~1%). Approximately 99% of AD cases are nonfamilial and late-onset, i.e., sporadic AD (SAD) (onset age >65 years old), and the pathophysiology of this disorder is not yet fully understood. APP is an extremely complex molecule that may be functionally important in its full-length configuration, as well as the source of numerous fragments with varying effects on neural function, yet the normal function of APP remains largely unknown. This article provides an overview of our current understanding of APP, including its structure, expression patterns, proteolytic processing and putative functions. Importantly, and for the first time, my recent data concerning its epigenetic regulation, especially in alternative APP pre-mRNA splicing and in the control of genomic rearrangements of the *APP* gene, are also reported. These findings may provide new directions for investigating the role of APP in neuropathology associated with a deficiency in the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGprt) found in patients with Lesch-Nyhan syndrome (LNS) and its attenuated variants (LNVs). Also, these findings may be of significance for research in neurodevelopmental and neurodegenerative disorders in which the APP gene is involved in the pathogenesis of diseases such as autism, fragile X syndrome (FXS) and AD, with its diversity and complexity, SAD in particular. Accurate quantification of various APP-mRNA isoforms in brain tissues is needed, and antisense drugs are potential treatments.

Keywords: Alzheimer's disease; autism; β-amyloid precursor protein; β-amyloid (Aβ) peptide; epigenetics; epistasis; fragile X syndrome; genomic rearrangenments; HGprt; Lesch-Nyhan syndrome.

DOI 10.1515/bmc-2014-0041
Received December 10, 2014; accepted January 22, 2015

***Corresponding author: Khue Vu Nguyen,** Department of Medicine, Biochemical Genetics and Metabolism, The Mitochondrial and Metabolic Disease Center, School of Medicine, University of California, San Diego, Building CTF, Room C-103, 214 Dickinson Street, San Diego, CA 92103, USA; and Department of Pediatrics, University of California, San Diego, School of Medicine, San Diego, La Jolla, CA 92093, USA, e-mail: kvn006@ucsd.edu

Introduction

The β-amyloid precursor protein (APP) belongs to a family of evolutionary and structurally related proteins. The human APP cDNA sequence was first cloned from a brain tissue library (1), and subsequently, a number of homologous APP family members were identified in a variety of mammalian and non-mammalian organisms. The APP family in mammals consists of three members: APP, the APP-like protein-1 (APLP1) and the APP-like protein-2 (APLP2). In non-mammals, it consists of such proteins as APL-1 in *Caenorhabditis elegans* and APPL in *Drosophila* (2–4). Human APP is a type-I transmembrane glycoprotein with a long N-terminal extracellular region and a short C-terminal cytoplasmic tail (5, 6). Human APP is best known as the precursor molecule that generates β-amyloid (Aβ) through its proteolysis. Aβ is a 39–42 amino acid peptide, the amyloid fibrillar form of which is the primary component of amyloid plaques found in the brains of individuals with Alzheimer's disease (AD) and Down syndrome (7). Aβ was identified by Glenner and Wong (8). The regulation of APP expression, the mechanisms of APP trafficking, post-translational modification and proteolytic cleavage are now well understood (9). The production of Aβ from APP, which is generally considered to be a key event in the pathogenesis of AD, has also been well studied (9). The importance of APP in AD clearly lies

in its role as a precursor to the Aβ peptide, which plays a central role in amyloid hypothesis (10). Since 1992, the amyloid cascade hypothesis has played a prominent role in explaining the etiology and pathogenesis of AD. It proposes that the deposition of Aβ is the initial pathological event in AD leading to the formation of senile plaques (SPs) and then to neurofibrillary tangles (NFTs), neuronal cell death and, ultimately, dementia (10). AD is currently classified by age at onset and genetic status (11). Sporadic AD (SAD) is characterized by later age at onset (onset age >65 years) and accounts for ~99% of AD cases. Familial AD, (FAD) accounting for ~1% of cases, is characterized by early age at onset (onset age <65 years) and a genetic component (11). In these cases, mutations in the *APP* gene and the *presenilin (PS)* genes *PS1* and *PS2* are known to be associated with FAD. In contrast to monogenic diseases, SAD exhibits numerous non-Mendelian anomalies that suggest an epigenetic component in the disease etiology. Furthermore, over recent years, data have illustrated that reciprocal interactions between APP and its various metabolites, including Aβ, can powerfully regulate key neural functions including cell excitability, synaptic transmission and neural plasticity (12). As a consequence, perturbations of some of these activities may contribute to AD pathogenesis and neurodegeneration in an Aβ-dependent or Aβ-independent manner (13). As such, it is important to continue to investigate the normal function of APP. Understanding its function will not only provide insights into the genesis of AD but may also prove vital in the development of an effective therapy. The present review focuses on the biomolecular and epigenetic aspects of human APP, beginning with an overview of APP including its structure, expression patterns, processing characteristics and putative functions, followed by its epigenetic regulation especially in alternative APP pre-mRNA splicing and in the control of genomic rearrangements of APP gene and, finally, concludes with a discussion and some future perspectives.

Human APP overview

APP structure

The human *APP* gene is located on chromosome 21 (21q21.2-3), spans approximately 240 kb and contains at least 18 exons (5, 6) (Figure 1A). Structurally, APP has features of an integral type-I transmembrane glycoprotein. The size of the putative binding domain at the N-terminus suggests that APP may act as a cell-surface receptor for a ligand (1), act as a growth factor (14) or bind to an extracellular matrix component such as proteoglycan (15). The APP-promoter sequence indicates that the *APP* gene belongs to the class of housekeeping genes. The promoter lacks typical TATA and CAAT boxes but contains consensus sequences for the binding of a number of transcription factors, including SP-1, AP-1 and AP-4 sites, a heat-shock control element and two Alu-type repetitive sequences (16–18). The presence of SP-1, AP-1, and AP-4 sites in the APP promoter regulates, the expression of proteins associated with cell proliferation and mitosis as well as cell differentiation and suggests that APP has a function related to cell growth or maturation. Structurally, APP can be divided into three domains: APP extracellular domain (EC) (exons 1–17: amino acid residues 1–699), APP transmembrane domain (TM) (exons 17–18: amino acid residues 700–723) and APP intracellular domain (IC) (exons 17–18: amino acid residues 724–770). Of interest, the Aβ sequence (exons 16–17: amino acid residues 672–713) is not conserved and is unique to APP. The encoded protein contains a large N-terminal extracellular domain that includes a signal-peptide sequence (SP) (amino acid residues 1–18), a cysteine-rich globular domain (E1) (exons 1–5: amino acid residues 18–190), an acidic domain, a Kunitz-type protease inhibitor (KPI) domain, a helix-rich domain (E2) (exons 9–14: amino acid residues 366–568) and part of the Aβ sequence, which extends into the transmembrane domain. The relatively short intracellular domain contains the C-terminus, which has some phosphorylation sites and a YENPTY-sorting motif (Figure 1B).

E1 domain and acidic region

The crystal structure of the E1 domain shows similarities to known cysteine-rich growth factors, and so it is termed 'growth factor-like domain' (GFLD) (14). The E1 domain is divided into two distinct regions, the heparin-binding domain (HBD1) and the copper/metal-binding domain (CuBD) (Figure 1B). HBD1 is composed of a single α-helix and an anti-parallel β-sheet, with a loop rich in basic residues (96–110) that bind to heparin (14, 15). Immediately adjacent to the HBD1 is a hydrophobic pocket, which could form either a protein-binding site or a dimerization site (14). Adjacent to the HBD1 is the copper/metal-binding domain, which contains a single α-helix and a short β-sheet. This region can bind several metal ions (19). The role of this domain is unclear, but it has been suggested that copper (II)-binding and reduction may be a principal function (20). On the C-terminal side of the E1 domain is an acidic region of unknown significance that is rich in glutamic acid and aspartic acid residues (Figure 1B).

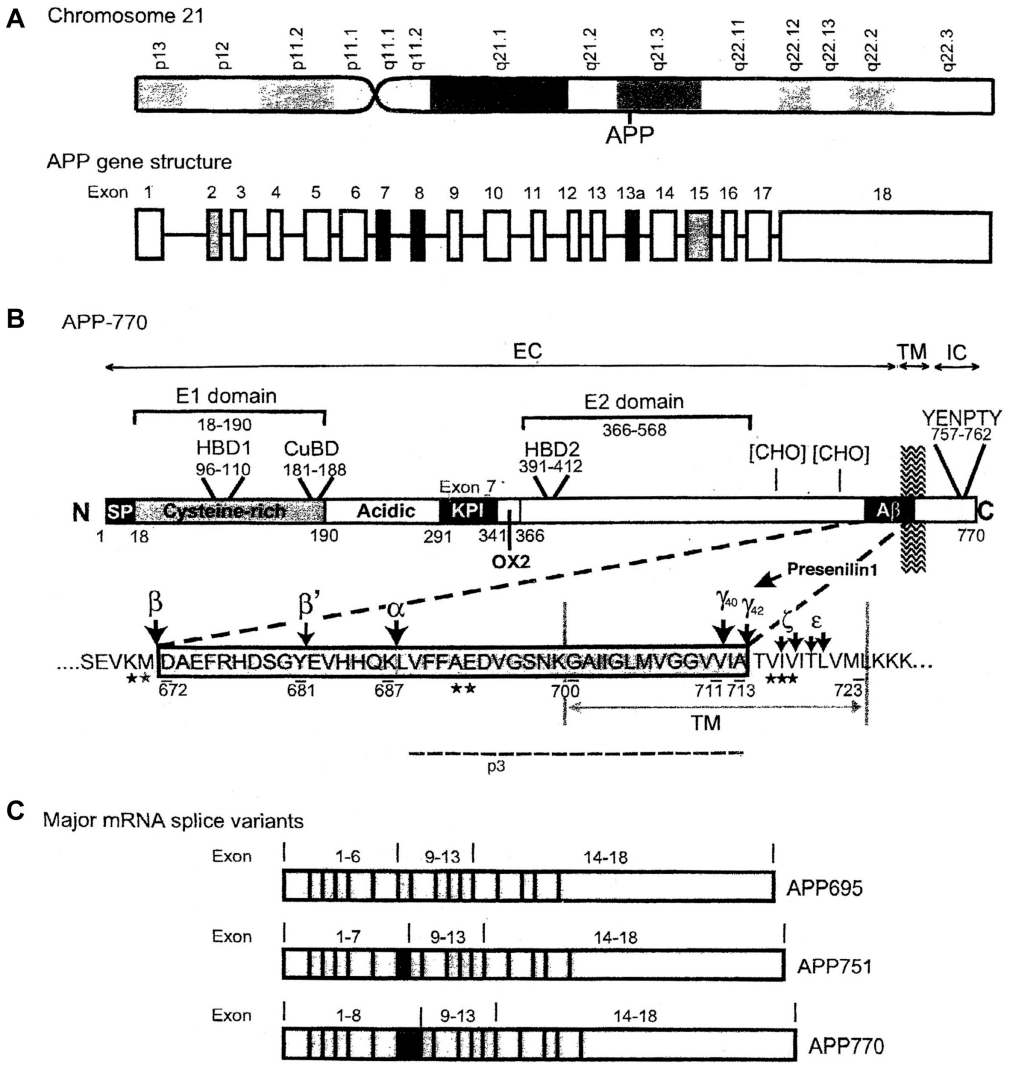


Figure 1: Structure of the amyloid precursor protein (APP): *APP* gene, APP_{770} structure and major mRNA splice variants.
(A) *APP* gene structure. The *APP* gene is located on chromosome 21q21.2-3, spans approximately 240 kb and contains at least 18 exons. Exon 13a (highlighted in black) denotes a novel exon containing a 59-nucleotide coding region and a 393-nucleotide untranslated region, as published by de Sauvage and Octage [see Ref. (6)]. (B) Protein structure (APP_{770}). APP can be divided into three domains: APP extracellular domain (EC), APP transmembrane domain (TM) and APP intracellular domain (IC). APP has an N-terminal signal peptide (SP); E1 domain with a heparin-binding domain (HBD1), a copper-binding domain (CuBD); acidic region; APP_{751} and APP_{770} contain a Kunitz protease inhibitor (KPI) domain located in exon 7 and an Ox-2 antigen domain; E2 domain with a second heparin-binding domain (HBD2); Between the E2 and Aβ regions are two potential N-linked glycosylation sites (CHO). The amino-acid sequence of the Aβ region is shown along with the secretase cleavages sites: BACE cleaves APP after Met_{671} (β) and Tyr_{681} (β′), whereas ADAM10 [best candidate α-secretase, see Ref. (44)] processes APP within the Aβ peptide sequence after Lys_{687} (α), thereby generating the p3 peptide. Gamma-secretase cleavage in the transmembrane region (TM) involves presenilin 1 (PS1) and generates Aβ peptides of mainly 40 and 42 amino acid residues long (γ_{40}) and (γ_{42}). PS-dependent proteolysis also occurs at other positions including the ε-site ($A\beta_{49}$) downstream of the γ-site proximal to the membrane-intracellular boundary (for details concerning the γ- ε- and ζ-sites, see text). Asterisks indicate the locations of mutations in APP causing early-onset of familial AD (FAD) (see AD Mutation Database, http://molgen-www.uia.ac.be/ADMutations). The intracellular (IC) C-terminal domain contains a YENPTY sorting motif. (C) Major mRNA splice variants. Differential mRNA splicing of exons 7, 8 (highlighted in black) can lead to the expression of 695, 751 and 770 amino acid isoforms.

KPI and Ox-2 antigen domains

Longer isoforms of APP (APP_{770} and APP_{751}) may contain a Kunitz-type protease inhibitor (KPI) domain located in exon 7 and an Ox-2 antigen domain (Figure 1B). APP isoforms containing the KPI domain are more commonly expressed in non-neuronal cells (21). KPI-containing APP isoforms are highly expressed in platelets, where they can

influence wound repair by regulating blood clotting serine proteases (22). As serine proteases are also implicated in neuronal cell growth (23), it is possible that KPI-containing APP isoforms regulate cell growth by inhibiting one or more of these proteases. Concerning the Ox-2 domain, this is an insert of 19 amino acid residues that is similar to a region of the Ox-2 antigen. The Ox-2 antigen is a lymphoid and neuronal cell-surface glycoprotein that has homology to Thy-1 and immunoglobulin light chains (24). As immunoglobulin loop domain are commonly found in cell-surface receptors and involved in cell-surface binding and recognition, it seems likely that the Ox-2 domain in APP has a similar function.

E2 domain

The E2 domain is an α-helix rich region that can readily dimerize (25) and may therefore be involved in APP self-association. Specially, X-ray analysis has revealed that the E2 domain of APP could form parallel or antiparallel dimers (26), and the latter structure implies that there is a potential to function in trans-cellular adhesion. Indeed, cell-culture studies support the homo-or hetero-dimers formation of APP family members, and trans-dimerization has been shown to promote cell-cell adhesion (27). It has been further demonstrated that heparin binding to the E1 or E2 region would induce the formation of APP dimerization (28). Downstream of the E1 and E2 regions, a 'RHDS' motif in the extracellular domain of APP within the Aβ sequence also appears to promote cell adhesion (Figure 1B). It is believed that this region acts in an integrin-like manner by homology to 'RGD' sequences (29). In this regard, it is interesting that APP co-localizes with integrins on the surface of axons and at sites of adhesion (30, 31). The E2 domain has also a heparin-binding site (HBD2) (32, 33) (Figure 1B) as well as a number of putative metal-binding sites that may hold the E2 domain in a rigid conformation (34). Between the E2 domain and the Aβ region are found two potential N-linked glycosylation sites (CHO) at residues 542 and 571 (Figure 1B).

Aβ, transmembrane domain and intracellular domain

The Aβ region on the C-terminal side of the E2 domain lies partly within the extracellular domain and partly within the transmembrane domain (Figure 1B). The Aβ peptides vary in size from 39 to 42 amino acids, and $A\beta_{1-42}$ aggregates more readily than the other molecules (35). A GxxxG sequence motif within the transmembrane domain has been implicated in homodimerization (36) and in cholesterol-binding (37) (Figure 1B). The intracellular domain contains a YENPTY sorting motif located between residues 757 and 762 of the APP_{770} isoform (Figure 1B). This YENPTY sorting motif interacts with several proteins containing phosphotyrosine binding-domains (PTB-domain), including Mint/X11 adaptor proteins. This motif is involved in the facilitation of clathrin-mediated endocytosis and is present in many tyrosine receptor kinases, non-receptor tyrosine kinases, low-density lipoprotein-receptor-related family proteins and integrins (38, 39). Consistent with this role, many studies have demonstrated that the YENPTY motif in APP is involved in the regulation of its trafficking and endocytosis (40–42).

APP expression

During transcription, alternative splicing generates APP mRNAs encoding several isoforms that range from 365 to 770 amino acid residues. The major expressed isoforms of APP have 695, 751 or 770 amino acid residues (Figure 1C). APP_{751} and APP_{770} contain a domain homologous to the Kunitz-type serine protease inhibitors (KPI) located in exon 7 of the extracellular sequences (Figure 1B and C), and these isoforms are commonly expressed in non-neuronal cells (21). APP_{695} isoform lacks the KPI domain (Figure 1C) and is predominantly expressed in neurons and accounts for the primary source of APP in brain (43). The raison and functional significance for this apparent tissue-specific alternative splicing is poorly understood.

APP processing

APP is processed in the constitutive secretory pathway and is post-translationally modified by N- and O-glycosylation, phosphorylation and tyrosine sulfation (44). Full-length APP is sequentially processed by at least three proteinases, termed α-, β- and γ-secretases (Figure 2). Cleavage by α-secretase or β-secretase within the luminal/extracellular domain results in the shedding of nearly the entire ectodomain to yield large soluble APP derivatives (called APPsα and APPsβ, respectively) and generation of membrane-tethered α- or β-carboxyl-terminal fragments (APP-CTFα and APP-CTFβ). The APP-CTFs are subsequently cleaved by γ-secretase to generate either a 3-kDa product (p3, from APP-CTFα) or Aβ (from APP-CTFβ), and the APP intracellular domain (AICD) (Figures 1B and 2). The major neuronal β-secretase is a transmembrane

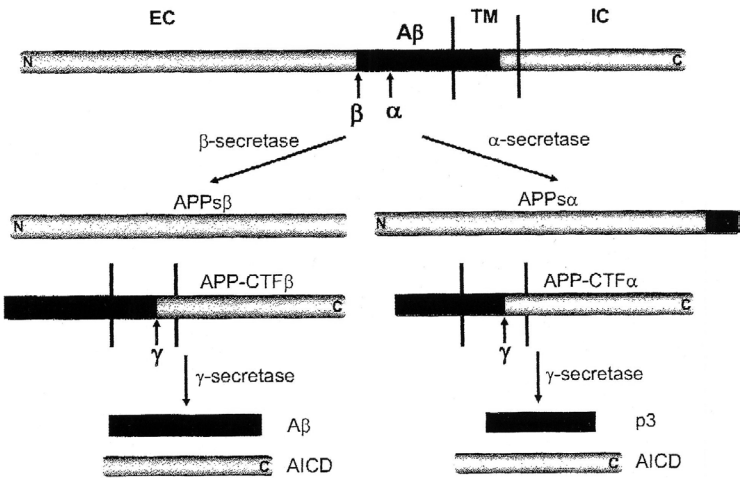


Figure 2: Schematic diagram of APP processing pathways (not drawn to scale).
The Aβ domain is highlighted in black. For simplicity, only one cleavage site is shown for each enzyme. EC, extracellular domain; TM, transmembrane domain; IC, intracellular domain.

aspartyl protease, termed BACE1 (β-site APP cleaving enzyme 1 or β-amyloid cleaving enzyme 1; also called Asp-2 and memapsin-2) (45–48). There is an alternative BACE (β′) cleavage site following Glu at position+11 of the Aβ peptide (49) (Figure 1B). In addition, there is a BACE2 homolog (β-site APP cleaving enzyme 2) that is expressed widely but does not appear to play a role in Aβ generation as it appears to cleave near the α-secretase site (50, 51). Whereas cleavage at the β-site is specific to BACE1, it was initially believed that a number of proteases, specifically members of the ADAM (a disintegrin and metalloprotease) family of proteases including ADAM9, ADAM10 and ADAM17, are candidates for the α-secretase (52). However, it should be noted that cleavage of APP by α-secretase processing only precludes the formation of an intact full length Aβ peptide. Although this latter event is commonly called the non-amyloidogenic pathway, that is unfortunately a bit of a misnomer because truncated Aβ (p3 peptide) from 17–42 (Figures 1B and 2) is also deposited in the brain of AD and Down syndrome patients (53, 54), indicating that shorter Aβ peptides starting at the α-secretase site may contribute to some aspects of AD-associated amyloid pathology (55, 56). Regarding γ-secretase cleavage that releases Aβ from the membrane, this activity is executed by a high molecular weight complex consisting of presenilin PS1 or PS2, nicastrin, anterior pharynx defective phenotype (APH1) and presenilin enhancer 2 (PEN2) (57, 58). Although these four proteins form the mature γ-secretase complex, it appears that the core γ-secretase activity resides within presenilin itself, functioning as an aspartyl protease (59–61). Gamma-secretase cleavage is a type of regulated intramembrane proteolysis (RIP), as cleavage occurs in the middle of the transmembrane domain (62). In addition to generating Aβ peptides of different lengths, RIP of APP is thought to occur as a series of cleavages, starting from the C-terminal end of the substrate and moving toward the N-terminal region of the transmembrane domain. These cleavage sites have been termed the γ- ε- and ζ-sites (62) (Figure 1B). In addition to the γ-cleavage that yields Aβ 40 and 42, PS-dependent proteolysis also occurs at other positions including the ε-site (Aβ 49) downstream of the γ-site proximal to the membrane-intracellular boundary (63–65). Recent data provide support for a sequential cleavage model in which ε-cleavage serves as the initial cutting site followed by ζ- and γ-processing within the membrane (65–67). Thus, the ε-cleavage of APP may represent the primary PS-dependent processing event. This is important because this cleavage releases AICD and more than 20 proteins have been reported to interact with AICD, including some that are necessary for AICD-dependent functions in signal transduction, apoptosis or modulation of cytoskeletal dynamics (68). It is also important to note that initially it was believed that the cleavage of APP is physiologically carried out by α-secretase, and it prevents amyloidogenesis in AD. In reality, this is an oversimplification, because only a fraction of the total pool of APP is cleaved by α-secretase in most cell types, leaving most of the APP protein intact. Furthermore, β- and γ-secretase processing of APP (at the N- and C-terminals of the Aβ sequence) also occur under physiological conditions; this indicates that all fragments of APP, including the Aβ peptide, are part of

normal physiology (44). It is also worth mentioning that none of the secretases have unique substrate specificity toward APP, and the cleavage of transmembrane proteins by an ADAM or BACE (ectodomain shedding) is commonly involved in the activation of a number of functional pathways. Ectodomain shedding by ADAMs is essential for the release of many cytokines and growth factor ligands, such as epidermal growth factor (EGF) (69). Additionally, ADAMs are involved in the ectodomain shedding of growth-factor receptors, such as human epidermal growth factor receptor 2 (70) and Notch (71). Ectodomain shedding by BACE is also likely to be required for the proper function of a number of proteins (72). For example, neuregulin-1 (NRG1) is cleaved by BACE1 and ADAM17 to release an ectodomain fragment that acts in a paracrine manner to stimulate myelination (73). Therefore, cleavage by ADAMs or BACE can potentially facilitate cellular signaling in a variety of ways, either by the release of growth factors or by the ligand-dependent activation of cellular receptors. RIP by γ-secretase is also a process involved in the normal function of many proteins. RIP can serve two general functions. First, it can remove the membrane-associated fragment that is produced by ectodomain shedding. Second, it can catalyze the production of intracellular signaling domains (62). Gamma-secretase has been reported to cleave more than 50 type-I membrane proteins in addition to APP (74). Apart from APP, the most well-known γ-secretase substrate is the developmental protein Notch, which is activated by γ-secretase cleavage (75, 76). Therefore, it is possible that γ-secretase cleavage may also be involved in the function of APP.

Putative functions of APP

Despite the large number of published studies on APP, there is still no clear consensus on the protein's function. There are many excellent reviews that summarize this area in detail but are otherwise beyond the scope of this article. Briefly, an analogy of the secondary structures and proteolytic processing profiles between Notch and APP predicts that APP could function as a cell-surface receptor similar to Notch (77). The E1 and E2 regions in the extracellular domain of APP have been shown to interact with extracellular matrix proteins and heparin sulfate proteoglycans (78), supporting its role in cell-substratum adhesion, cell-cell adhesion, dimerization, ligand-binding and metal-binding. A number of publications have pointed to an important role of the APP extracellular domain in neurite outgrowth and synaptogenesis, both as a full-length protein and as a secreted molecule (APPs) following ectodomain-shredding. Furthermore, a role of APP in cell signaling and apoptosis via AICD has been also documented (68). Thus, APP may exert these activities in both autocrine and paracrine fashions. Because levels of APPs have been reported to be reduced in individuals with AD (79), the possibility has been raised that the loss of the trophic activity of APPs, in concert with a reduction of growth factors in the brain, may contribute to neurodegeneration in AD. One of the mechanisms that regulate APP function is likely to be protein phosphorylation. APP can be phosphorylated at multiple sites in both extracellular and intracellular domains (80). In particular, protein phosphorylation by protein kinase C (PKC) has been shown to play a pivotal role in the control of APP metabolism and amyloid formation (80). Among different sites of phosphorylation, the phosphorylation at the threonine$_{668}$ residue (Thr$_{668}$, numbering for the APP$_{695}$ isoform, i.e., Thr$_{743}$ for the APP$_{770}$ isoform) in the APP intracellular domain has received the most attention to date. This phosphorylation has been implicated in regulating APP localization to the growth cones and neurites (81, 82). Significantly, the Thr$_{668}$ phosphorylated APP is shown to be preferentially transported to the nerve terminals (83), and the Thr$_{668}$ phosphorylated APP fragments are increased in AD, but not in control subjects (84), raising the possibility that this phosphorylation event may contribute to AD pathogenesis by regulating Aβ generation in neurons. A recent work provided a possible link between Thr$_{668}$ phosphorylation and APP processing through the prolyl isomerase Pin1. The authors showed that Pin1 binds to the phosphorylated Thr$_{668}$-Pro motif and promotes the isomerization of the proline residue (85). This leads to a conformational change of the APP intracellular domain and alteration of APP processing and Aβ production. Furthermore and interestingly, the APPThr$_{688}$ phosphorylation and adaptor protein interaction may be functionally coupled. Biochemical and structural studies indicate that Thr$_{688}$ phosphorylation results in a conformational change by which it negatively regulates APP binding to Fe65 and reduces the stability of the APP intracellular domain (86). Overall, these findings lend support for an important role of Thr$_{668}$ phosphorylation and Fe65 in regulating APP dynamics. Concerning the two other human proteins that have a high degree of homology to APP, but do not contain the Aβ sequence, APLP1 and APLP2, and in regard to the pathology of AD, although APLP1 and APLP2 do not produce the toxic Aβ peptide, their roles in functioning separately from, but in support of, APP suggest that they may play a role in the development of the disease. Indeed, it was reported that both APLP1

and APLP2, like APP, formed transcriptionally active triple protein complexes with Mint3 and transcriptional co-activators Taz and Yap to activate transcription of target genes, and the complex formation was regulated by the γ-secretase cleavage of APLP1 and APLP2. The presence of Mint1 instead of Mint3 in the complex prevented its translocation to the nucleus. APLP1 displayed much lower transactivation levels compared to APP and APLP2. These results indicate that all these three human APP family members are capable of activating gene transcription via Mint3-Taz and Mint3-Yap (87).

To understand the *in vivo* function of APP and its processing products, the *in vivo* gain-and loss-of-function phenotypes associated with the APP family of proteins in model systems (*C. elegans*, *Drosophila* and mice) were also investigated (88). In *C. elegans*, although the cloning of the *C. elegans* APP homolog APL-1 was published in 1993, to date there has been no report describing the APL-1 null mutant. It was reported that, by using an RNA-interference (RNAi) method, worms treated with APL-1 RNAi exhibited a defect in pharyngeal pumping, and this phenotype was shared with Fe65 homolog-1 (Feh-1)-treated worms, suggesting that these two proteins act in the same pathway, possibly through direct physical interactions. Mutation in Feh-1 resulted in a reduction of acetylcholinesterase gene expression. These findings taken together support a conserved role of the APP family of proteins in the cholinergic pathway, possibly in a Fe65-dependent manner. For *Drosophila*, it was reported that mutants lacking APPL were viable and fertile but exhibit subtle behavioral defects that can be partially rescued by human APP, demonstrating functional conservation. Subsequent analysis revealed that these mutant flies show reduced synaptic bouton numbers at the neuromuscular junction, and that this activity appears to require the formation of a complex with the cell adhesion molecule fasciclin and *Drosophila* Mint/X11. Consistent with a role of APPL in synapse development, ectopic over-expression of APPL leads to satellite bouton formation at the *Drosophila* neuromuscular junction, and this activity requires the APP YENPTY domain where adaptor protein interaction takes place. In mice, homozygous APP-deficient mice are viable and fertile. However, the mutant animals were smaller (15–20% less body weight) than age-matched controls and exhibited decreased locomotor activity and forelimb grip strength, indicating compromised neuronal or muscular function. In addition, the majority of the mice show reactive gliosis, suggesting undefined neuronal damage in brain activity. The relatively subtle phenotype of the APP-deficient mice indicated that the presence of other APP family members may compensate for the loss of APP. Indeed, whereas mice with individual deletion of APP, APLP1 and APLP2 are viable and fertile, APP/APLP2 and APLP1/APLP2 double-knockout mice showed early postnatal lethality. Intriguingly, the APP/APLP1 double-null mice are viable, revealing a property of APLP2 that is uniquely required when APP or APLP1 is absent. Although the neuromuscular junction of APP or APLP2 single-null mice did not show overt structural abnormalities, the APP/APLP2 double-knockout animals exhibited poorly formed neuromuscular junctions with reduced apposition of pre- and postsynaptic elements of the junctional synapses. The number of synaptic vesicles at the presynaptic terminals was also reduced. Mice deficient in all three APP family members (APP/APLP1/APLP2 triple-knockout mice) were lethal in the early postnatal period. Analysis of these APP/APLP1/APLP2 triple-knockout mice revealed that the majority of the animals showed cortical dysplasia suggestive of neuronal migration abnormalities and partial loss of cortical Cajal Retzius cells. In sum, the loss-of-function studies present a convincing picture that members of the APP gene family play essential roles in the development of the peripheral and central nervous systems relating to synapse structure and function, as well as in neuronal migration or adhesion.

In conclusion, it is clear that APP undergoes complex regulation and is important for neuronal and synaptic function in both central and peripheral nervous systems. This may involve the APP extracellular domain, the APP intracellular domain, the Aβ sequence or, indeed, cross communication among these motifs. It is therefore reasonable to speculate that the misregulation of APP could contribute to neuronal and synaptic impairment occurring in AD in an Aβ-dependent or Aβ-independent manner. As such, a more complete understanding of AD pathogenesis will likely require greater insights into the physiological function of APP.

Epigenetic regulation of APP

Regulation of alternative APP pre-mRNA splicing

As mentioned in the Introduction, human APP is the best known as the precursor molecule whose proteolysis generates β-amyloid (Aβ), a 39-42 amino acid peptide whose amyloid fibrillar form is the primary component of amyloid plaques found in the brains of individuals with Alzheimer's disease (AD) and Down syndrome (7, 9). Current therapeutics designed to treat AD have not been

successful in effectively treating the progressive nature of cognitive decline observed in patients. Cholinesterase inhibitors donepezil, rivastigmine and galantamine, huperzine-A, and N-methyl-D-aspartate antagonist memantine are approved drugs available for AD; however, these drugs fail to treat the underlying cause of neurodegeneration and only provide modest short-term symptomatic relief (89). One of the underlying reasons for the failure of treatment measures is the lack of knowledge pertaining to the epigenetic, environmental, and mechanistic drivers of neurodegeneration. Many studies have focused on determining the genetic contributions to AD. In FAD, autosomal dominant mutations in APP, PS1, and PS2 [all genes involved in the production of Aβ, see Ref. (11)] are mainly responsible for the early-onset form of the disease. Recently, the triggering receptor expressed on myeloid cells 2 (TREM2) was designed as another rare monogenetic candidate for early onset of disease risk (90). However, these mutations in FAD represent a very small percentage of cases (~1%), and ~99% of cases are SAD (11). Multiple studies conducted to determine disease-causative loci have revealed that AD is highly complex and heterogeneous in nature, susceptibility loci vary according to gene penetrance, ethnicity, geography, environment and sample size of various studies; therefore, identifying genes responsible for SAD is highly challenging (91–93). Linkage and genome-wide association studies (GWAS) have mapped regions in chromosomes 9, 10 and 12 that are associated with AD risk; several genes, such as apolipoprotein E (APOE), BACE1 and BACE2, for example, are thought to be prime candidates to confer risk because of their role in pathways associated with Aβ biosynthesis and deposition (94). However, approximately 300 genes have been implicated without strong causative evidence to increase risk for AD (92). Therefore, nongenetic factors, such as epigenetic modifications, may also be causative and currently the subject of intense research. Epigenetics is the study of changes in gene expression and/or chromatin structure and cell function caused by mechanisms other than changes in the underlying DNA sequence (95). This field is an important area of investigation because epigenetic modifications may explain differential regulation of AD risk genes and genomic regions without changes to their DNA sequence and, therefore, undetected in genetic studies. These modifications can occur on DNA molecules (mainly on cytosine bases at cytosine-guanine: CpG sites) or on histone proteins, which make up the fundamental structure of chromatin (96, 97). Modifications to histones include methylation, acetylation, phosphorylation, ubiquitination, sumoylation, etc., and more than 100 different modification residues have been characterized (98). Variations in promoter sequences can alter gene expression directly by altering a transcription-binding site or indirectly by changing the organization of chromatin. Promoter variants with effects on the transcriptional activity of certain human genes and in the regulation of alternative pre-mRNA splicing have been identified, and genetic association studies have suggested that some of these variants may be disease risk factors (99, 100). Recently, noncoding RNAs (ncRNAs), such as long noncoding RNAs (LncRNAs) and microRNAs (miRNAs), among other regulatory RNA molecules, have been shown to play an epigenetic role in the regulation of genes by various mechanisms, including recruiting DNA methyltransferases and chromatin modifiers to their targets, inhibiting translation of mRNA, and in the degradation or stability of mRNA by sequence complementarity with their targets (101). Epigenetic modifications are therefore central facilitators of the nexus between genes and the environment. The role of epigenetic modifications has been studied in various fields of biology, especially in developmental and cancer biology; erroneous regulation of epigenetic modifications has been linked to many developmental deficiencies, including neurogenesis, and in the formation and progression of cancer (102, 103). The role of epigenetic modifications in neurological disease has also been studied; erroneous regulation of epigenetic modifications results in a number of neurological disorders such as Rett syndrome, mental retardation, autism and AD (104–107). Recently, I performed a study (108) searching for a link between APP and Lesch-Nyhan syndrome (LNS), a neurogenetic disorder of purine metabolism in which the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGprt, EC. 2.4.2.8; MIM 308000) is defective. Complete or severe deficiency of HGprt enzyme activity leads to LNS (MIM 300322). Classical features of LNS include hyperuricemia and its sequelae (gout, nephrolithiasis and tophi), intellectual impairment and self-injurious behavior (109). Partial deficiency of HGprt enzyme activity (MIM 300323) leads to its attenuated variants (LNVs). LNV is characterized by the consequences of overproduction of uric acid and a variable spectrum of neurological manifestations, without the manifestations of self-injurious behavior (110, 111). The etiology of LNS and LNV involves a mutation of the *HPRT1* gene, which is on the long arm of the X chromosome (Xq26.1), and it contains nine exons and eight introns (110, 111). Because the *HPRT1* gene is on the X chromosome, males are affected and females in the families are at risk for being carriers of the mutation. A major unsolved question is how the loss of HGprt enzyme function affects the brain to cause the neurobehavioral syndrome in LNS and

its attenuated variants (LNVs). To address this issue, a link between LNS and the aberrant basal ganglia function, including the dysfunction of dopaminergic pathways, was reported (112, 113). However, the mechanism by which features of LNS result from impaired purine metabolism is still not well understood. It was also documented that adhesion of HGprt-deficient neuroblastoma as well as fibroblasts from patients with LNS exhibited dramatically enhanced adhesion compared to control cells (114) and could have consequences for the maturation of the central nervous system, as seen in the smaller brain size of LNS and LNVs children (115–117). It was also reported that HGprt-deficiency was accompanied by aberrations in a variety of pathways known to regulate neurogenesis or to be implicated in neurodegenerative disease, including the canonical Wnt/β-catenin and the AD/presenilin signaling pathways (118). Furthermore, it was also documented that the *HPRT1* gene regulated multiple developmental and metabolic pathways of murine embryonic stem cell neuronal differentiation, and the neural aberrations of HGprt-deficiency could result from the combinatorial effects of these multi-system metabolic errors (119). As some of these aberrations are also found in forms of AD and Huntington's disease, these authors predicted that some of the systems defects play similar neuropathogenic roles in diverse neurodevelopmental and neurodegenerative diseases (119). A role for APP related to cell-cell or cell-substrate adhesion and important for brain morphology and highly coordinated brain functions such as memory and learning has been suggested (12, 88). Hence, the APP pathway is possibly implicated in the development of LNS and LNVs. In an attempt to search for a link between APP and LNS (108), I have examined the APP-mRNA profile and the genomic APP-DNA in fibroblasts from normal subjects and HGprt-deficient LNS and LNVs patients. Interestingly, during this study (108), I identified for the first time, in fibroblasts from normal subjects as well as from LNS and LNVs patients, several APP-mRNA isoforms encoding divers APP protein isoforms ranging from 120 to 770 amino acids (with or without mutations and/or deletions) (Table 1, see Supplementary Material for additional details about the experimental procedures). There was no mutation found in the 18 exons and flanking intronic sequences of genomic APP-DNA from all fibroblasts. Divers APP-mRNA isoforms with or without mutations and/or deletions have been found in fibroblasts of all subjects (normal control as well as LNS and LNVs patients), and it is therefore conceivable that changes in epigenetic regulation caused by genetic and environmental factors, as well as life events and aging, could cause alterations in the regulation of alternative APP pre-mRNA splicing and result in an imbalance between different APP-mRNA isoforms. Some of these isoforms may be disease risk factors. It is the first time that the real profile of APP-mRNA isoforms accounting for epigenetic mechanisms in the regulation of alternative APP pre-mRNA splicing has been shown. Here, it is important to note that epigenetic modifications, due to gene-gene interactions (epistasis) (120), could affect the regulation of alternative APP pre-mRNA splicing in favor of APP-mRNA isoforms responsible for the disease. Epistasis is important, ubiquitous and has become a hot topic in complex disease genetics, such as AD, schizophrenia, autism, type-2 diabetes, sporadic breast cancer, sickle-cell anemia, etc., in recent years and even common for determining phenotypes for a number of rare Mendelian diseases such as cystic fibrosis, Hirschsprung disease, etc. (121). However, the data supporting epistasis in complex human diseases are emerging slowly. This is due to different difficulties that we face in detecting and characterizing epistasis, such as challenges of modeling non-linear interactions, and in the interpretation of results (120, 121). My present results accounted for epigenetic mechanisms in the regulation of alternative APP pre-mRNA splicing and may provide, therefore, a unique integrative framework for the pathologic diversity and complexity of neurodevelopmental and neurodegenerative disorders in which the APP gene is involved in the pathogenesis of the diseases such as autism, AD and, especially SAD. Although there is no experimental evidence at present proving the direct link between LNS/LNVs and APP, based on the results summarized in Table 1, it is conceivable that in LNS, epigenetic modifications due to epistasis between mutated *HPRT1* and *APP* genes could affect the regulation of alternative APP pre-mRNA splicing in favor of APP-mRNA isoforms responsible for the modifications of brain anatomy and the neurobehavioral syndrome observed in LNS and LNVs. The severity of the affection would depend on how mutations ultimately alter the interactions between mutated *HPRT1* and *APP* genes. The type of mutation and its location in the HPRT1 gene is therefore an important factor for provoking disease (LNS or LNV), not only through its effect on residual HGprt enzyme activity but also through its effect on interactions between mutated *HPRT 1* and *APP* genes. For the same type of mutation in the *HPRT1* gene, the response to epigenetic modifications due to epistasis may be different from one patient to another and this could explain the manifestation of different clinical phenotypes from different patients and also from different affected family members (111, 122, 123).

Table 1: Isoforms of APP and mutations/deletions.

Samples[a]	Isoforms	Mutations and/or deletions
1	APP_{770}	No mutation
	APP_{770}	Mutation in exon 5: c.622T>C, p.V208A
	APP_{237}	Deletion starting after 102 bp of the 5' end of exon 5 followed by a complete deletion of exons 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 104 bp of the 5' end of exon 17. Mutation in exon 2: c.135A>G, p.N46D.
	APP_{168}	Deletion starting after 93 bp of the 5' end of exon 3 followed by a complete deletion of exons 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 59 bp of the 5' end of exon 17. No mutation.
7	APP_{770}	Mutations in exon 6: c.751G>A, p.G251D; exon 7: c.979A>G, p.N327S.
	APP_{770}	Mutations in exon10: c.1249A>G, p.E417G; exon 11: c.1429T>C, p.I477T; exon 13: c.1657C>T, p.A553V.
	APP_{207}	Deletion starting after 49 bp of the 5' end of exon 3 followed by complete deletion of exons 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15. Mutations in exon 1: c.21C>T, p.L8F; exon 3: c.268A>G, p.Q90R.
	APP_{120}	Deletion starting after 27 bp of the 5' end of exon 3 followed by a complete deletion of exons 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 138 bp of the 5' end of exon 17. No mutation.
13	APP_{770}	No mutation.
	APP_{770}	Mutation in exon 12: c.1563delA, p.K522fs531X in exon 13.
	APP_{751}	Mutation in exon 15: c.1930C>T, p.P644L.
	APP_{751}	Mutations in exon 12: c.1557C>T, p.P520S; c.1570C>T, p.A524V; exon 16: c.2062T>C, p.L688S.
	APP_{216}	Deletion starting after 33 bp of the 5' end of exon 3 followed by a complete deletion of exons 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 11 bp of the 5' end of exon 15. No mutation.
	APP_{168}	Deletion starting after 63 bp of the 5'end of exon 3 followed by a complete deletion of exons 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 30 bp of the 5' end of exon 17. No mutation.
14	APP_{770}	No mutation.
	APP_{770}	Mutation in exon 2: c.135A>G, p.N46D.
	APP_{334}	Deletion starting after 9 bp of the 5' end of exon 6 followed by a complete deletion of exons 7, 8, 9, 10, 11, 12, 13, 14, 15 and 15 bp of the 5' end of exon 16. No mutation.
	APP_{193}	Deletion starting after 42 bp of the 5' end of exon 3 followed by a deletion of 209 bp of the 5' end of exon 14. Complete deletion of exons 4, 5, 6, 7, 8, 9, 10, 11, 12, 13. Deletion in exon 2: c.199delC, p.Q74fs86X in exon 3. Mutation in exon 3: c.242G>T, p.Q81H.
	APP_{175}	Deletion starting after 132 bp of the 5' end of exon 2 followed by a complete deletion of exons 3,4,5,6,7,8,9,10, 11,12,13,14,15, and 10 bp of the 5' end of exon 16. Mutation in exon 18: c.2265G>A, p.G756S.
15	APP_{770}	No mutation.
	APP_{770}	Mutations in exon 9: c.1215A>G, p.M406V; exon 10: c.1380T>A, p.D427E; exon 16: c.2050A>G, p.H684R.

[a]Sample #1 is normal subject, control; Samples #7 and #13 are LNS-affected male patients; Samples #14 and #15 are LNV-affected male patients.

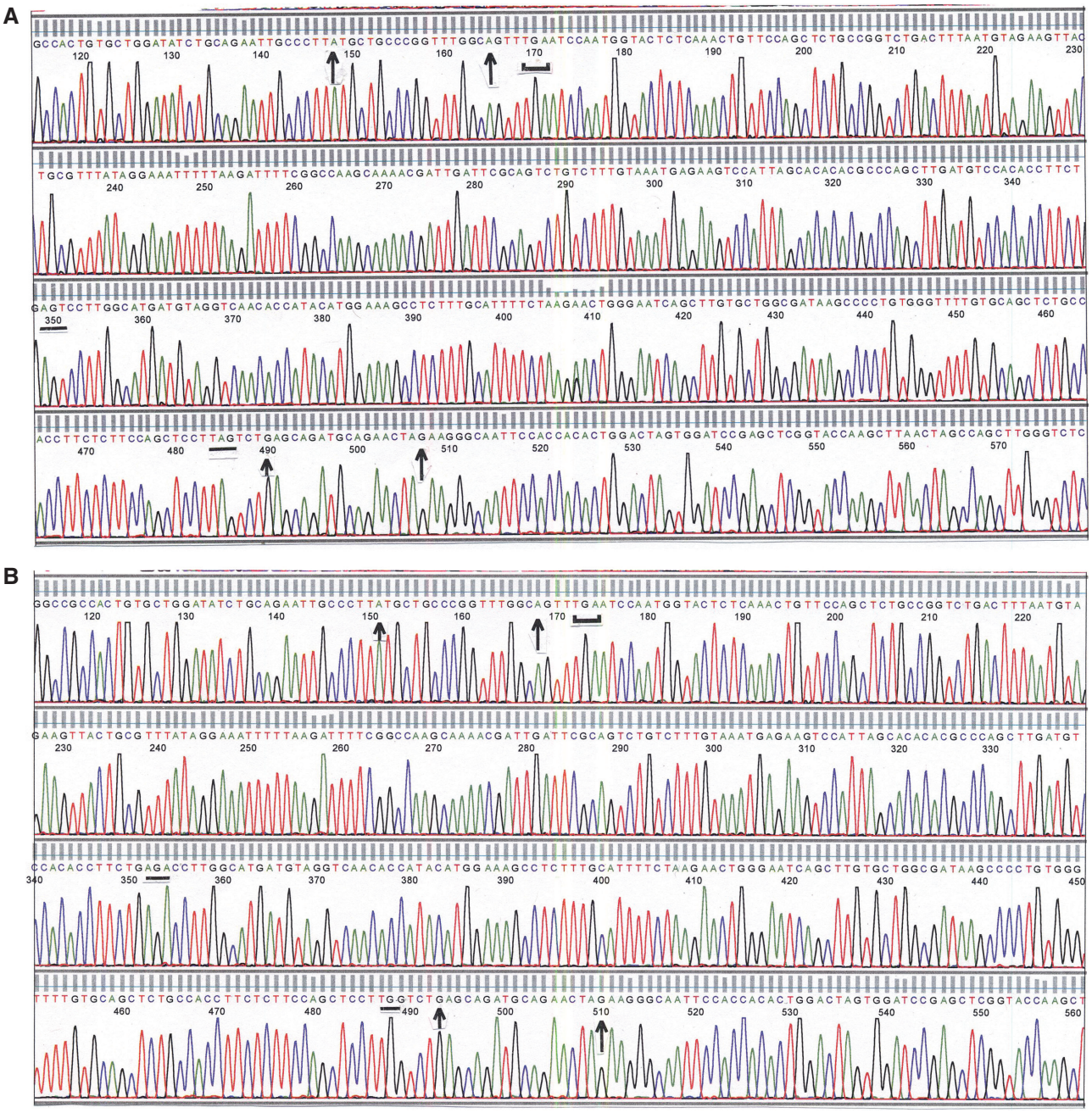
Control of genomic rearrangements of APP gene

In the past 15 years, it has become evident that higher-order genomic architectural features can lead to a susceptibility to DNA rearrangements that are frequent causes of disease (124). Genomic rearrangements describe mutational changes in the genome such as duplication, deletion, insertion, inversion and translocation that are different from the traditional Watson-Crick base pair alterations (125). Genomic rearrangements can represent polymorphisms that are neutral in function, or they can convey phenotypes via diverse mechanisms, including changing the copy number (that is, copy number variation or CNV) of dosage-sensitive genes, disrupting genes, creating fusion genes or other mechanisms (125). The pathological conditions caused by genomic rearrangements are collectively defined as genomic disorders (125–127). CNV is a major source of genetic variation among humans. CNV can convey clinical phenotypes, including genomic disorders, sporadic diseases and complex human traits such as autism (128), schizophrenia (129) and mental retardation (130). Thus, uncovering the mechanisms underlying CNV formation has tremendous implications for the diagnostics of CNV-associated diseases. Typically, the term 'genomic rearrangements' is only used to describe gross DNA changes ranging from thousands to sometimes millions of base pairs that can cover clusters of different genes (125). Three major mechanisms have been proposed for rearrangements in the human genome: non-allelic homologous recombination (NAHR), non-homologous end-joining (NHE) and the Fork Stalling and Template Switching (FoSTeS) models (125). Recently, Chen et al. (131) proposed the serial replication slippage (SRS) model to explain the 'smaller' DNA rearrangements (between 21 bp and up to 10 kb), including duplications, deletions,

insertions and inversions, collected in the Human Gene Mutation Data base (HGMD). The SRS model proposed for small gene mutations shares some general features with the FoSTeS model proposed for the larger rearrangements. Both models assume serial replication slippage, and both stress the importance of the genomic architectural elements, such as palindromic DNA, stem-loop structures, repeats and so on, that may facilitate the initial stalling of the replication fork. Whereas the SRS model assumes that replication slippage occurs on closely adjacent sites (possibly inside the same replication fork) and causes DNA rearrangements of small sizes, the FoSTeS model emphasizes that the template switch can occur over long distances (120 kb to 550 kb observed to date) to another replication fork (given the spatial closeness of the two forks) and cause DNA rearrangements on a much larger scale (124). Recently, in the research work of searching for a link between APP and LNS (108), I have also identified the presence of genomic rearrangements of *APP* gene. For the first time, three APP-mRNA isoforms resulted from DNA rearrangements of *APP* gene with a deletion followed by an insertion (INDELS) via the SRS mechanism leading to non-functional APP or loss of APP. This is due to the early translational stops which resulted in transcript instability c.19_2295delC_{166}TG...TTT_{2442}insG_{166}TT...GAG<u>T</u>CC... CTT<u>A</u>GTC...TCT_{489}, p.Leu7Valfs*2 for sample #7, LNS; c.19_2295delC_{166}TG...TTT_{2442}insG_{169}TT...GAG<u>A</u>CC... CTT<u>G</u>GTC...TCT_{492}, p.Leu7Valfs*2 for sample #15, LNV; and c.16_2313delG_{163}CA...TAG_{2460}insG_{84}CC...CAT_{616}, p.Leu7Hisfs*45 for sample #14, LNV. The underlined letters T, A, and A, G indicate the difference in nucleotides in the sequence of 324 bp inserted in these two APP-cDNA (Figure 3A, B and C, see Supplementary Material for additional details about the experimental procedures). Indeed, as shown in Figure 3A and B, there was a deletion between exon 1 and exon 18 of the APP gene starting after nucleotide A_{165} or A_{168} in exon 1 of APP-cDNA and substituted by an insertion of 324 bp in which there were five Opal stop codons TGA located after nucleotides T_{168}, C_{210}, T_{276}, A_{300}, T_{330} and one Amber stop codon TAG located after nucleotide T_{483} for sample #7, LNS (Figure 3A), and five Opal stop codons TGA located after nucleotides T_{171}, C_{213}, T_{279}, A_{303}, T_{333} for sample #15, LNV (Figure 3B), respectively. In fact, the sequence of 324 bp inserted in these two APP-cDNA is identical except for nucleotides T_{351} (in triplet AGT, see horizontal bar mark) and A_{485} (in triplet TAG, see horizontal bar mark) in sample #7 (Figure 3A) and A_{354} (in triplet AGA, see horizontal bar mark) and G_{488} (in triplet TGG, see horizontal bar mark) in sample #15 (Figure 3B). Also, as shown in Figure 3C, there was a deletion in exon 1 of the APP gene starting after nucleotide G_{83} in exon 1 of APP-cDNA and substituted by an insertion of 533 bp in which there were five Opal stop codons TGA located after nucleotides G_{118}, C_{260}, C_{281}, A_{284}, C_{365} and two Amber stop codons TAG located after nucleotides A_{239}, C_{353} for sample #14, LNV. Here, all other exons of the APP gene were totally excluded. Only the first stop codons presented in the inserted sequence of all three APP-cDNA are shown in Figure 3A, B and C because only the first is a real stop codon, the others are part of the 3′unstranslated region (UTR) and will not function as stop codon (see horizontal bracket marks). As a result, APP produced from these three APP-mRNA isoforms could not function. Early translational stops can lead to transcript instability, leading to the loss of APP. As a consequence, there is a disease risk. Here, experiments conducted on the controls (normal subjects) have not shown any clones of the APP-mRNA isoforms with the genomic rearrangements comparable to those observed in LNS and LNVs patients. Given the small size of the DNA rearrangements deduced from the three APP-mRNA isoforms isolated in this study, the SRS model is likely the one responsible for the present genomic rearrangements of the *APP* gene. The complex gene rearrangements caused by the SRS mechanism for diverse mutations found in different genes, such as *GALNS, HPRT1, IDS, MECP2, OFD1, SALL1*, etc., have been reported (131). Duplication of the APP locus resulting from the genomic rearrangements has been documented [see Ref. (132) and the AD Mutation Database, http://molgen-www.uia.ac.be/ADMutations]. Recently, epigenetic changes controling genomic rearrangements of the IgH locus has been reported (133). It is therefore conceivable that changes in epigenetic regulation caused by genomic rearrangements of the *APP* gene could result in an imbalance between different APP-mRNA isoforms, and some of these isoforms may be disease risk factors. Epistasis between mutated HPRT1 and *APP* genes could be one of the factors of epigenetic modifications responsible for genomic rearrangements of the *APP* gene.

Discussion and perspectives

AD is the most common form of dementia and represents a progressive, degenerative brain disorder that affects memory, behavior and emotion. To date, the pathophysiology of this disorder is not yet fully understood. APP is central to understanding AD pathogenesis due to its genetic, biochemical and neuropathological connections with AD. Firstly, APP is the source of Aβ, a major component of SPs in AD brains (7, 9). Secondly, genetic mutations of *APP* cause FAD [see Ref. (11) and AD Mutation Database].



(**Figure 3:** Continued)

Furthermore, the mutation spectrum extended to APP locus duplications, underscoring the importance of the *APP* gene dosage in AD [see Ref. (132) and AD Mutation Database]. In the case of trisomy of chromosome 21 in Down syndrome, AD neuropathology develops universally due to an extra copy of the *APP* gene (7). Current theories on the pathophysiology of AD are mainly based on genetic and neuropathological findings pointing toward aberrant processing of APP and tau as central molecular events. Although most patients are SAD, it is mainly the research performed on FAD patients that provided valuable insights into disease pathogenesis. Mutations in the genes encoding for APP and presenilins (PS1 and PS2), which are involved in APP processing, have been shown to induce FAD. The presenilin and APP mutations found in FAD patients appear to result in increased production of $A\beta_{42}$, which is probably the primary neurotoxic species involved in the pathogenesis of the disease (134). In FAD, mutations in APP itself or in the presenilins can shift the cleavage site to favor the γ-secretase site and, in particular, to favor

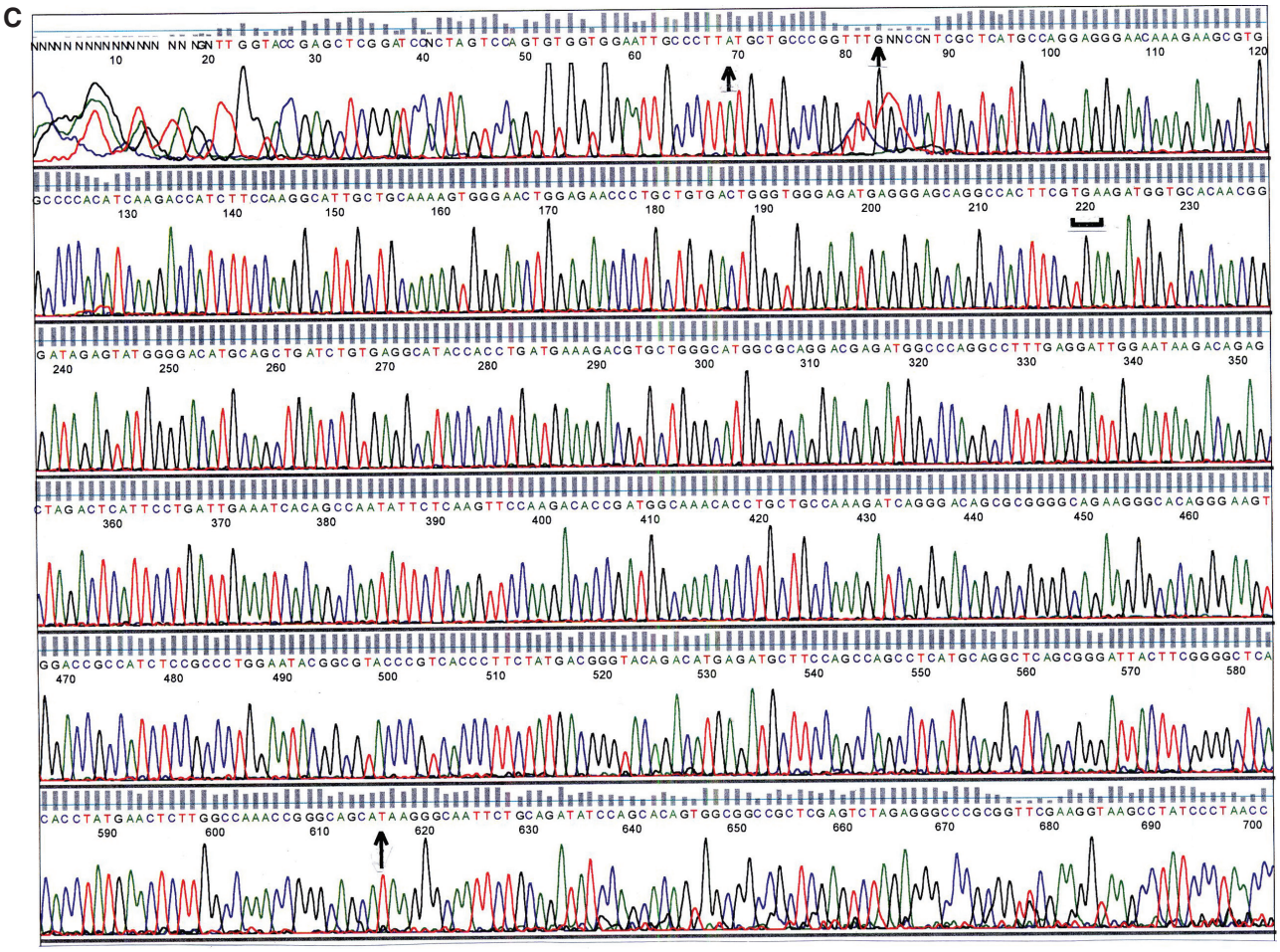


Figure 3: Automated direct APP-cDNA sequence analysis for screening of insert into the pcDNA™3.1/V5-His-TOPO®vector.
(A) Sample #7, LNS: Chromatogram of the APP-cDNA sequence read from left to right (5′→3′) showed the nucleotide A_{148} of the ATG start codon of exon 1 of APP gene (↑); the beginning of the deletion and substituted by an insertion of 324 bp between exon 1 and exon 18, located after nucleotide A_{165} (↑); the first Opal stop codon TGA, located after nucleotide T_{168} (see horizontal bracket mark); the presence of nucleotides T_{351} (in triplet AGT, see horizontal bar mark) and A_{485} (in triplet TAG, see horizontal bar mark); the nucleotide G_{490} of the GAG start codon of exon 18 (↑); the nucleotide G_{507} of the Amber stop codon TAG end codon of exon 18 at the end of APP-cDNA (↑). (B) Sample #15, LNV: Chromatogram of the APP-cDNA sequence read from left to right (5′→3′) showed the nucleotide A_{151} of the ATG start codon of exon 1 of APP gene (↑); the beginning of the deletion and substituted by an insertion of 324 bp between exon 1 and exon 18, located after nucleotide A_{168} (↑); the first Opal stop codon TGA, located after nucleotide T_{171} (see horizontal bracket mark); the presence of nucleotides A_{354} (in triplet AGA, see horizontal bar mark) and G_{488} (in triplet TGG, see horizontal bar mark); the nucleotide G_{493} of the GAG start codon of exon 18 (↑); the nucleotide G_{510} of the Amber stop codon TAG end codon of exon 18 at the end of APP-cDNA (↑). (C) Sample #14, LNV: Chromatogram of the APP-cDNA sequence read from left to right (5′→3′) showed the nucleotide A_{69} of the ATG start codon of exon 1 of APP gene (↑); the beginning of the deletion and substituted by an insertion of 533 bp in exon 1, located after nucleotide G_{83} (↑); the first Opal stop codon TGA, located after nucleotide G_{218} (see horizontal bracket mark); the nucleotide T_{616} at the end of APP-cDNA (↑).

increased production of the toxic $A\beta_{42}$ peptide over the shorter, less toxic $A\beta_{40}$ peptide. The PS1 may in fact be the γ-secretase itself or a necessary cofactor in γ-secretase activity (134). The identification of mutations in APP and the presenilins in FAD not only suggests a common mechanism through which mutations in these genes may exert their deleterious effects (i.e., increased production or decreased clearance of $A\beta_{42}$ and formation of a protein aggregate, the amyloid plaque) but also provides evidence of a direct role of the $A\beta_{42}$ peptide and presenilins in the pathogenesis of the disease (134). Despite the rarity of APP and presenilin mutations, their functional evaluation in transfected cells, transgenic animals and human plasma identified an elevation in Aβ levels, increased $A\beta_{42}$ / $A\beta_{40}$ ratio or fibrillogenesis (135), which constituted the cornerstone of the amyloid cascade hypothesis (10). Accordingly, increases in the toxic forms of Aβ lead to a cascade of events – including inflammation, synaptic loss, ionic

imbalance, and abnormal phosphorylation of proteins (including tau) – culminating in cell death and underlying clinical dementia. Neurofibrillary tangles (NFTs) are formed by hyperphosphorylation of the tau protein, as a result of mutations or age-related alterations in the expression of the microtubule-associated protein tau (MAPT) gene (10). The pathophysiology of AD is therefore characterized by complex interplay between factors in aging and aberrant processing of both APP and tau (107). Additionally, another type of genetic variation that has been underrepresented in genetic studies of AD but gained attention over the last few years, given its contribution to phenotypic diversity and complex diseases, is the CNVs (125). CNVs are implicated in a number of neurodegenerative disorders including AD where *APP* duplications result in early-onset autosomal-dominant AD [see Ref. (132) and AD Mutation Database]. Since the proposal of the amyloid cascade hypothesis (10), the AD research community has been split into two broad groups, those that support the amyloid cascade hypothesis and those that do not: (a) SPs and NFTs may be developed independently, and (b) SPs and NFTs may be the products rather than causes of neurodegeneration in AD (136, 137). Moreover, randomized clinical trials that tested drugs or antibodies targeting components of the amyloid pathway have been inconclusive (138). As important as the rare early-onset forms of FAD have been for understanding of the pathogenesis of the disease, the majority of patients have SAD, in which no mutation in the *APP* or *presenilin* genes has been identified. The best-established gene known to influence SAD is the gene encoding apolipoprotein E (APOE), a constituent of the low-density lipoprotein particle (94, 135). Three variants of the gene and the protein are found in human populations and result from changes in single amino acids in apolipoprotein E (referred to as the APOE $\varepsilon 2$, $\varepsilon 3$ and $\varepsilon 4$ alleles). APOE is a component of SPs, binds Aβ, can influence neuritic plaque formation in transgenic mouse models of AD in an isoform-specific fashion and is thought to contribute to both Aβ clearance and deposition in the brain. *In vitro* and *in vivo* studies also suggest a role for APOE in isoform-specific synaptogenesis and cognition, neurotoxicity, tau hyperphosphorylation, neuro-inflammation and brain metabolism, although these non-Aβ-related mechanisms require further investigation. Unlike APP and the presenilins mutations that are fully penetrant (causal), APOE $\varepsilon 4$ is a genetic risk factor that is neither necessary nor sufficient for the development of AD. The molecular mechanisms by which the variations of APOE alleles alter the age at onset and, therefore, the lifetime risk of AD are unknown. The odds that APOE $\varepsilon 3/\varepsilon 4$ genotype carriers have AD is estimated to be two to four times greater than that of APOE $\varepsilon 3/\varepsilon 3$ carriers, according to population-based association studies in subjects of European origin. The odds ratio increases to approximately 6 to 30 in the APOE $\varepsilon 4/\varepsilon 4$ genotype carriers. Although there is evidence of a risk effect of APOE $\varepsilon 4$ in non-Europeans, the estimated effect sizes are smaller with less consistent results in African-American and Hispanic subjects, which may suggest different underlying genetic or environmental factors or both for these ethnic groups. The effect of APOE $\varepsilon 4$ appears to be age-dependent, with the strongest effect observed before age 70. Nonetheless, APOE $\varepsilon 4$ does not account for all genetic variations in AD (11). The use of APOE as a diagnostic or predictive factor in clinical practice is not warranted (135). It is interesting to note herein that the first AD patient, Auguste D., in whom the disease was discovered, had early-onset AD (she died at age 56 years). Neuropathological examination of her brain revealed numerous NTFs and amyloid plaques. Her APOE genotype ($\varepsilon 3/\varepsilon 3$) did not predispose her to AD, and she did not have the mutations at codons 692, 693, 713 and 717 in exon 17 of *APP* gene (139). In addition, she did not carry the N141I mutation in PS2 characteristic of AD in Volga Germans (140). These findings do not preclude a different mutation in *PS2* or in the *APP* gene, or a mutation in *PS1*. The etiology and biological pathogenesis initiating the early-onset of neuritic plaques and neurofibrillary tangles in Auguste D. remains a mystery today, just as it was for Alzheimer more than 100 years ago. A number of associations of the disease with variants of genes other than APOE have also been reported but remain to be confirmed and are the subject of ongoing research (92, 94). In any way, many chronic neurological and psychiatric diseases may have at least a partial epigenetic etiology. For example, AD genetics alone does not fully explain the pathogenesis of the disease. Therefore, nongenetic factors, such as epigenetic phenomena, or environmental stimuli, such as infections (bacteria, viruses), radioactivity, pesticides, hormones, heavy metal, etc., as well as multiple modifiable vascular risk factors, such as mid-life hypertension, hypercholesterolaemia, diabetes, smoking, etc., nutritional factors, emotional and social factors are likely to contribute to the development of AD (141). Furthermore, there is also evidence supporting a role for epistasis in the etiology of complex traits, such as AD, schizophrenia, autism, type-2 diabetes, sporadic breast cancer, etc., in which there were no single-gene effects. A gene does not function by itself, but rather acts with other genes in a network, to influence complex traits (120, 121). Recently, it has also become evident that higher-order genomic architectural features can lead to a susceptibility to DNA rearrangements that are frequent causes of disease (124). Duplication of the *APP* locus resulting from genomic

rearrangements has been documented [see Ref. (132) and the AD Mutation Database], and epigenetic changes controlling genomic rearrangements of the IgH locus has been reported (133). The SAD cases are thus of a multifactorial nature, and they are likely to involve complex gene-environment and gene-gene interactions. However, to date, there are only suggestions about the influences of epigenetic modifications and epistasis in susceptibility to AD and the concept of epigenetics in the pathophysiology of AD but no real experimental results. In the present review, for the first time, the real profile of APP-mRNA isoforms accounting for epigenetic mechanisms in the regulation of alternative APP pre-mRNA splicing (108) due to epigenetic modifications and/or epistasis as well as for epigenetic control of genomic rearrangements of the *APP* gene has been shown. As a result of epigenetic regulation, different isoforms of APP-mRNA (with or without mutations and/or deletions) can exist, and the most abundant one quantitatively is decisive for the normal status or disease risk. For example, apart from APP-mRNA, isoforms with missense mutation(s), such as APP_{770} (sample #1, normal subject, control), APP_{751} (sample #13, LNS), APP_{770} (sample #14, LNV), $2APPs_{770}$ (sample #7, LNS), APP_{751} (sample #13, LNS) and APP_{770} (sample #15, LNV), in which the consequence of disease risk is unknown and needs to be checked experimentally using expression vectors, all APP-mRNA isoforms with premature stop codon and/or with partial and/or complete deletions of several exons resulted from genomic rearrangements of the *APP* gene, such as the three APP-mRNA isoforms for sample #7, LNS (Figure 3A), sample #15, LNV (Figure 3B) and sample #14, LNV (Figure 3C), or resulted from alternative APP pre-mRNA splicing, such as APP_{237}, APP_{168} (sample #1, normal subject, control), APP_{207}, APP_{120} (sample #7, LNS), APP_{216}, APP_{168} (sample #13, LNS), APP_{334}, APP_{193}, APP_{175} (sample #14, LNV), (see Table 1) are disease risks because these exons of the *APP* gene are responsible for different putative functions of APP as mentioned in the human APP overview section. An accurate quantification of various APP-mRNA isoforms in brain tissues for detection of initial pathological changes or pathology development is therefore needed. Indeed, although the current major focus is on using DNA to identify disease genes, mutations and translocations, or foreign genes as infectious agents, the quantification of various specific mRNA in cells and tissues is an attractive field in diagnostic molecular pathology because the concentrations of each specific mRNA are different in normal and disease states. These concentrations also change rapidly in response to various clinical treatments (122). Mutations in the *APP* and *PS* genes that are involved in FAD (with or without SPs and/or NFTs), occurring in less than 1% of all AD cases, are only the specific cases of AD. Here, epistasis between *APP* and *PS* genes should be also considered. The type of mutation and its location in *APP* and/or *PS* genes should be therefore an important factor for provoking disease. In these forms of AD, alterations in the regulation of alternative APP pre-mRNA splicing could cause an imbalance between different APP-mRNA isoforms in favor of the ones in which there is increased production of the toxic $A\beta_{42}$ peptide over the shorter, less toxix $A\beta_{40}$ peptide. This could explain the different mutations in *PS* affecting γ-secretase structure or function in multiple ways (142). Based on my present findings, it appears that according to the type of APP-mRNA isoform responsible for the pathology development, the pathogenesis of AD could occur in an Aβ-dependent or Aβ-independent manner. SPs and NTFs may consequently be developed independently, and SPs and NTFs are the products rather than the causes of neurodegeration in AD. These findings may provide new directions, by performing an accurate quantification of various APP-mRNA in brain tissues, for the research of early-onset AD from the first AD patient, Auguste D., in whom the disease was discovered. Recently, an increased secreted soluble APP derivative from the cleavage by α-secretase (APPsα fragment) in the plasma of severely autistic patients has been reported (143–146). These authors speculated that overproduction of APPsα may contribute to the state of brain overgrowth implicated in autism and FXS. It is, however, documented that APPsα is neuroprotective, neurotrophic and regulates cell excitability and synaptic plasticity (12). Here, changes in epigenetic regulation caused by genetic, epigenetic and/or epistasis could affect the regulation of alternative APP pre-mRNA splicing in favor of APP-mRNA isoforms in which there is an overproduction of APPsα (aberrant non-amyloidogenic processing of APP via α-secretase pathway, see Figure 2) that may contribute to autism and FXS phenotypes. An accurate quantification of various APP-mRNA isoforms in brain tissues of autistic and FXS patients may be therefore useful for investigating the role of APP in these neurodevelopmental disorders. It has also been suggested that treatment with metabotropic glutamate receptor 5 (mGluR5) antagonists may help repress APP-mRNA translation and reduce secretion of APPsα in autism and FXS (145).

Once the APP-mRNA isoform responsible for the disease is identified, one of the potential treatments for the disease may include the inhibition or repression of translation into APP isoform from this APP-mRNA isoform [by using antisense drugs such as a specific antisense oligonucleotide or a chemical analogue, see Refs. (147–149)]. Antisense drugs are a new generation of gene-silencing

therapeutic agents with potential for targeted causal treatment of as yet incurable diseases. In principle, antisense technology is supposed to prevent protein production from a targeted gene. The exact mechanism by which this occurs remains uncertain. Proposed mechanisms include triplex formation, blocking RNA splicing, preventing transport of the mRNA antisense complex into the cytoplasm, increasing RNA degradation or blocking the initiation of translation. When the genetic sequence of a particular gene is known to be causative of a particular disease, it is possible to synthesize a strand of nucleic acid (DNA, RNA or a chemical analogue) that will bind to the mRNA produced by that gene and inactivate it, effectively turning that gene 'off'. This is because mRNA has to be single-stranded for it to be translated. Alternatively, the strand might be targeted to bind a splicing site on pre-mRNA and modify the exon content of an mRNA. This synthesized nucleic acid is termed an 'anti-sense' oligonucleotide because its base sequence is complementary to the gene's mRNA, which is called the 'sense' sequence. This technology may be used to treat various conditions including cancer, diabetes, neurodevelopmental and neurodegenerative disorders and hypertension, as well as autoimmune and cardiovascular diseases. Antisense drugs are potentially potent, selective and well-tolerated drugs and are becoming more widely accepted as potential therapeutics for various diseases (147–149).

Conclusions

In conclusion, although LNS is a rare disease, self-injurious behavior is a manifestation of many neuropsychiatric disorders. Therefore, there is much value in studying a rare disease that might ultimately shed light on more common disorders. LNS was the first neurogenetic disorder for which the responsible enzyme was identified (109–111). Close to the 50th anniversary of its first description in two siblings (109), we continue to learn much from HGprt-deficiency, an enzyme defect that substantially modifies brain anatomy (115–117) and cause the neurobehavioral syndrome observed in LNS and LNVs (109–111). Finally, through a massive research effort over the last 2 decades, it has now become clear that APP and its fragments play diverse roles in development and cell growth, cell adhesion, intercellular communication, signal transduction, nuclear signaling and structural and functional plasticity. However, the physiological function of APP is not well understood as yet. Understanding of its function will not only provide insights into the genesis of the disease but may also prove vital in the development of an effective therapy. APP is an important molecular hub at the center of interacting pathways, and therefore it is not surprising that altered APP processing may affect brain function through a host of altered cellular and molecular events. My findings may provide new directions not only for investigating the role of APP in neuropathology associated with HGprt-deficiency in LNS and LNVs patients but also for research in neurodevelopmental and neurodegenerative disorders in which the APP gene is involved in the pathogenesis of diseases, such as autism, FXS and AD, with its diversity and complexity, and especially SAD.

Acknowledgments: This work was supported by grants from the Lesch-Nyhan Syndrome Children's Research Foundation and the Harold A. and Madeline R. Jacobs Fund at The San Diego Foundation. I am grateful to the patients and their families for agreeing to participate in this study.

List of abbreviations

Aβ	β-amyloid peptide
AD	Alzheimer's disease
ADAM 9	a disintegrin and metalloprotease-9
ADAM 10	a disintegrin and metalloprotease-10
ADAM 17	a disintegrin and metalloprotease-17
AICD	β-amyloid precursor protein intracellular domain
AP-1	activator protein 1
AP-4	activator protein 4
APH1	anterior pharynx defective 1
APL-1	β-amyloid precursor-like protein 1 in *Caenorhabditis elegans*
APLP1	β-amyloid precursor-like protein 1 in mammals
APLP2	β-amyloid precursor-like protein 2 in mammals
APOE	apolipoprotein E
APP	β-amyloid precursor protein
APP-CTFα	APP carboxyl-terminal fragment released from APP following the cleavage by α-secretase
APP-CTFβ	APP carboxyl-terminal fragment released from APP following the cleavage by β-secretase
APPL	β amyloid precursor-like protein in *Drosophila*
APPsα	soluble APP fragment released from APP following the cleavage by α-secretase
APPsβ	soluble APP fragment released from APP following the cleavage by β-secretase
BACE1	β-site APP cleaving enzyme 1
BACE2	β-site APP cleaving enzyme 2
CNV	copy number variation
CuBD	copper/metal-binding domain
EC	extracellular domain
EGF	epidermal growth factor
FAD	familial Alzheimer's disease
Feh-1	Fe65 homolog-1

FoSTeS	fork stalling and template switching
FXS	fragile X syndrome
GALNS	galactosamine (N-acetyl)-6-sulfatase gene
GFLD	growth factor-like domain
GWAS	genome-wide association study
HBD1	heparin-binding domain 1
HBD2	heparin-binding domain 2
HGMD	human gene mutation data base
HGprt	hypoxanthine-guanine phosphoribosyltransferase
HPRT1 gene	hypoxanthine phosphoribosyltransferase 1 gene
IC	intracellular domain
IDS	iduronate 2-sulfatase gene
INDELS	deletion followed by an insertion
KPI	Kunitz protease inhibitor
LncRNAs	long noncoding RNAs
LNS	Lesch-Nyhan syndrome
LNVs	Lesch-Nyhan variants
MAPT	microtubule-associated protein tau
MECP2	methyl CpG-binding protein 2 gene
mGluR5	type 5 metabotropic glutamate receptor
MIM	Mendelian inheritance in man
Mint	Munc18 interacting protein, also known as X11
miRNAs	microRNAs
NAHR	non-allelic homologous recombination
ncRNAs	noncoding RNAs
NFTs	neurofibrillary tangles
NHE	non-homologous end-joining
NRG1	neuregulin-1
OFD1	oral-facial-digital syndrome 1 gene
Ox-2	orexin receptor type 2
PEN2	presenilin enhancer 2
Pin1	peptidyl-prolyl cis-trans isomerase NIMA-interacting 1
PKC	protein kinase C
PS	presenilin
PS1	presenilin-1
PS2	presenilin-2
PTB-domain	phosphortyrosine binding-domain
RNAi	RNA-interference
SAD	sporadic Alzheimer's disease
SALL1	spalt-like transcription factor 1 gene
SP	signal peptide
SP-1	specificity protein 1
SPs	senile plaques
SRS	serial replication slippage
TAZ	transcriptional co-activator with PDZ-binding motif
Thy-1	thymocyte antigen 1
TREM2	triggering receptor expressed on myeloid cells 2
UTR	unstranslated region
YAP	Yes-associated protein

References

1. Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Muller-Hill B. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 1987; 325: 733–6.
2. Muller UC, Zheng H. Physiological functions of APP family proteins. Cold Spring Harb Perspect Med 2012; 2: a006288.
3. Wasco W, Bupp K, Magendantz M, Gusella JF, Tanzi RE, Solomon F. Identification of a mouse brain cDNA that encodes a protein related to the Alzheimer disease-associated amyloid β protein precursor. Proc Natl Acad Sci USA 1992; 89: 10758–62.
4. Wasco W, Gurubhagavatula S, Paradis MD, Romano DM, Sisodia SS, Hyman BT, Neve RL, Tanzi RE. Isolation and characterization of APLP2 encoding a homologue of the Alzheimer's associated amyloid β protein precursor. Nat Genet 1993; 5: 95–100.
5. Yoshikai S, Sasaki H, Doh-ura K, Furuya H, Sakaki Y. Genomic organization of the human-amyloid β-protein precursor gene. Gene 1990; 87: 257–63.
6. Yoshikai S, Sasaki H, Doh-ura K, Furuya H, Sakaki Y. Genomic organization of the human-amyloid β-protein precursor gene. Gene 1991; 102: 291–2.
7. Master CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer's disease and Down's syndrome. Proc Natl Acad Sci USA 1985; 82: 4245–9.
8. Glenner GG, Wong CW. Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. Biochem Biophys Res Commun 1984; 122: 1131–5.
9. Thinakaran G, Koo EH. Amyloid precursor protein trafficking, processing, and function. J Biol Chem 2008; 283: 29615–9.
10. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. Science 1992; 256: 184–5.
11. Bettens K, Sleegers K, Van Broeckhoven C. Current status on Alzheimer disease molecular genetics: from past, to present, to future. Hum Mol Genet 2010; 19: R4–11.
12. Turner PR, O'Connor K, Tate WP, Abraham WC. Roles of amyloid precursor protein and its fragments in regulating neural activity, plasticity and memory. Prog Neurobiol 2003; 70: 1–32.
13. Chen J, Wang M, Turko IV. Quantification of amyloid precursor protein isoforms using quantification concatamer internal standard. Anal Chem 2013; 85: 303–7.
14. Rossjohn J, Cappai R, Feil S, Henry A, McKinstry WJ, Galatis D, Hesse L, Multhaup G, Beyreuther K, Masters CL, Parker MW. Crystal structure of the N-terminal, growth factor-like domain of Alzheimer amyloid precursor protein. Nat Struct Biol 1999; 6: 327–31.
15. Small DH, Nurcombe V, Reed G, Clarris H, Moir R, Beyreuther K, Masters CL. A heparin-binding domain in the amyloid protein precursor of Alzheimer's disease is involved in the regulation of neurite outgrowth. J Neurosci 1994; 14: 2117–27.
16. Salbaum JM, Weidemann A, Lemaire HG, Masters CL, Beyreuther K. The promoter of Alzheimer's disease amyloid A4 precursor gene. EMBO J 1988; 7: 2807–13.
17. Izumi R, Yamada T, Yoshikai S, Sasaki H, Hattori M, Sakaki Y. Positive and negative regulatory elements for the expression of the Alzheimer's disease amyloid precursor-encoding gene in mouse. Gene 1992; 112: 189–95.
18. Quitschke WW, Goldgaber D. The amyloid β-protein precursor promoter. A region essential for transcriptional activity contains a nuclear factor binding domain. J Biol Chem 1992; 267: 17362–8.
19. Bush AI, Multhaup G, Moir RD, Williamson TG, Small DH, Rumble B, Pollwein P, Beyreuther K, Masters CL. A novel zinc (II) binding site modulates the function of the β A4 amyloid protein precursor of Alzheimer's disease. J Biol Chem 1993; 268: 16109–12.

20. Multhaup G, Schlicksupp A, Hesse L, Beher D, Ruppert T, Masters CL, Beyreuther K. The amyloid precursor protein of Alzheimer's disease in the reduction of copper (II) to copper (I). Science 1996; 271: 1406–9.
21. Rohan de Silva HA, Wickenden C, Jen LS, Wilkinson SL, Patel AJ. Cell-specific expression of β-amyloid precursor protein isoform mRNAs and proteins in neurons and astrocytes. Brain Res Mol Brain Res 1997; 47: 147–56.
22. Van Nostrand WE, Schmaier AH, Farrrow JS, Cunningham DD. Platelet protease nexin-2/amyloid β-protein precursor. Possible pathologic and physiologic functions. Ann NY Acad Sci 1991b; 640: 140–4.
23. Wang H, Reiser G. Thrombin signaling in the brain: the role of protease-activated receptors. Biol Chem 2003; 384: 193–202.
24. Clark MJ, Gagnon J, Williams AF, Barclay AN. MRC OX-2 antigen: a lymphoid/neuronal membrane glycoprotein with a structure like a single immunoglobulin light chain. EMBO J 1985; 4: 113–8.
25. Xue Y, Lee S, Wang Y, Ha Y. Crystal structure of the E2 domain of amyloid precursor protein-like protein 1 in complex with sucrose octasulfate. J Biol Chem 2011; 286: 29748–57.
26. Wang Y, Ha Y. The X-ray structure of an antiparallel dimer of the human amyloid precusor protein E2 domain. Mol Cell 2004; 15: 343–53.
27. Soba P, Eggert S, Wagner K, Zentgraf H, Siehl K, Kreger S, Lower A, Langer A, Merdes G, Paro R, Masters CL, Muller U, Kins S, Beyreuther K. Homo- and heterodimerization of APP family members promotes intercellular adhesion. EMBO J 2005; 24: 3624–34.
28. Dahms SO, Hoefgen S, Roeser D, Schlott B, Guhrs KH, Than ME. Structure and biochemical analysis of the heparin-induced E1 dimer of the amyloid precursor protein. Proc Natl Acad Sci USA 2010; 107: 5381–6.
29. Ghiso J, Rostagno A, Gardella JE, Liem L, Gorevic PD, Frangione B. A 109-amino-acid C-terminal fragment of Alzheimer's-disease amyloid precursor protein contains a sequence, -RHDS-, that promotes cell adhesion. Biochem J 1992; 288: 1053–9.
30. Storey E, Spurck T, Pickett-Heaps J, Beyreuther K, Masters CL. The amyloid precursor protein of Alzheimer's disease is found on the surface of static but not activity motile portion of neurites. Brain Res 1996; 735: 59–66.
31. Yamazaki T, Koo EH, Selkoe DJ. Cell surface amyloid β-protein precursor colocalizes with β I integrins at substrate contact sites in neural cells. J Neurosci 1997; 17: 1004–10.
32. Multhaup G. Identification and regulation of the high affinity binding site of the Alzheimer's disease amyloid protein precursor (APP) to glycosaminoglycans. Biochimie 1994; 76: 304–11.
33. Clarris HJ, Cappai R, Heffernan D, Beyreuther K, Masters CL, Small DH. Identification of heparin-binding domains in the amyloid precursor protein of Alzheimer's disease by deletion mutagenesis and peptide mapping. J Neurochem 1997; 68: 1164–72.
34. Dahms SO, Konnig I, Roeser D, Guhrs KH, Mayer MC, Kaden D, Multhaup G, Than ME. Metal binding dictates conformation and function of the amyloid precursor protein (APP) E2 domain. J Mol Biol 2012; 416: 438–52.
35. Snyder SW, Ladror US, Wade WS, Wang GT, Barrett LW, Matayoshi ED, Huffaker HJ, Krafft GA, Holzman TF. Amyloid-β aggregation: selective inhibition of aggregation in mixtures of amyloid with different chain lengths. Biophys J 1994; 67: 1216–28.
36. Munter LM, Voigt P, Harmeier A, Kaden D, Gottschalk KE, Weise C, Pipkorn R, Schaefer M, Langosch D, Multhaup G. GxxxG motif within the amyloid precursor protein transmembrane sequence are critical for the etiology of Aβ42. EMBO J 2007; 26: 1702–12.
37. Barrett PJ, Song Y, Van Horn WD, Hustedt EJ, Schfer JM, Hadziselimovic A, Beel AJ, Sanders CR. The amyloid precursor protein has a flexible transmembrane domain and binds cholesterol. Science 2012; 336: 1168–71.
38. Bonifacino JS, Traub LM. Signals for sorting of transmembrane proteins to endosomes and lysosomes. Annu Rev Biochem 2003; 72: 395–447.
39. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2010; 141: 1117–34.
40. Lai A, Sisodia SS, Trowbridge IS. Characterization of sorting signals in the β-amyloid precursor protein cytoplasmic domain. J Biol Chem 1995; 270: 3565–73.
41. Perez RG, Soriano S, Hayes JD, Ostaszewski B, Xia W, Selkoe DJ, Chen X, Stokin GB, Koo EH. Mutagenesis identifies new signals for β-amyloid precursor protein endocytosis, turnover, and the generation of secreted fragments, including Aβ42. J Biol Chem 1999; 274: 18851–6.
42. Ring S, Weyer SW, Kilian SB, Waldron E, Pietzik CU, Filippov MA, Herms J, Buchholz C, Eckman CB, Korte M, Wolfer DP, Muller UC. The secreted β-amyloid precursor protein ectodomain APPs alpha is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice. J Neurosci 2007; 27: 7817–26.
43. Sisodia SS, Koo EH, Hoffman PN, Perry G, Price DL. Identification and transport of full-length amyloid precursor protein in rat peripheral nervous system. J Neurosci 1993; 13: 3136–42.
44. De Strooper B, Annaert W. Proteolytic processing and cell biological functions of the amyloid precursor protein. J Cell Sci 2000; 113: 1857–70.
45. Vassa R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, Citron M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 1999; 286: 735–41.
46. Yan R, Bienkowski MJ, Shuck ME, Miao H, Tory MC, Pauley AM, Brashier JR, Stratman NC, Mathews WR, Buhl AE, Carter DB, Tomasselli AG, Parodi LA, Heinrikson EL, Gurney ME. Membrane-anchored aspartyl protease with Alzheimer's disease β-secretase activity. Nature 1999; 402: 533–7.
47. Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M, Dovey HF, Frigon N, Hong J, Jacobson-Croak K, Jewett N, Keim P, Knops J, Lieberburg I, Power M, Tan H, Tatsuno G, Tung J, Schenk D, Seubert P, Suomensaari SM, Wang S, Walker D, Zhao J, McConlogue L, John V. Purification and cloning of amyloid precursor protein β-secretase from human brain. Nature 1999; 402: 537–40.
48. Cai H, Wang Y, McCarthy D, WEn H, Borchelt DR, Price DL, Wong PC. BACE1 is the major β-secretase for generation of Aβ peptides by neurons. Nat Neurosci 2001: 4: 233–4.
49. Huse JT, Liu K, Pijak DS, Carlin D, Lee VMY, Doms RW. β-secretase processing in the trans-Golgi network preferentially generates truncated amyloid species that accumulate in Alzheimer's disease brain. J Biol Chem 2002; 277: 16278–84.
50. Solans A, Estivill X, De La Luna S. A new aspartyl protease on 21q22.3, BACE2, is highly similar to Alzheimer's amyloid precursor protein β-secretase. Cytogenet Cell Genet 2000; 89: 177–84.

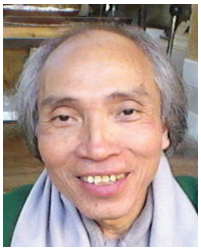
51. Yan R, Munzner JB, Shuck ME, Bienkowski MJ. BACE2 functions as an alternative α-secretase in cells. J Biol Chem 2001; 276: 34019–27.
52. Lichtenthaler SF. Alpha-secretase in Alzheimer's disease: molecular identity, regulation and therapeutic potential. J Neurochem 2011; 116: 10–21.
53. Gowing E, Roher AE, Woods AS, Cotter RJ, Chaney M, Little SP, Ball MJ. Chemical characterization of Aβ 17-42 peptide, a component of diffuse amyloid deposits of Alzheimer disease. J Biol Chem 1994; 269: 10987–90.
54. Lalowski M, Golabek A, Lemere CA, Selkoe DJ, Wisniewski HM, Beavis RC, Frangione B, Wisniewski T. The "nonamyloidogenic" p3 fragment (amyloid β17-42) is a major constituent of Down's syndrome cerebellar preamyloid. J Biol Chem 1996; 271: 33623–31.
55. Wei W, Norton DD, Wang X, Kusiak J. Aβ 17-42 in Alzheimer's disease activates JNK and caspase-8 leading to neuronal apoptosis. Brain 2002; 125: 2036–43.
56. Szczepanik AM, Rampe D, Ringheim. Amyloid-β peptide fragments p3 and p4 induce pro-inflammatory cutokine and chemokine production *in vitro* and *in vivo*. J Neurochem 2001; 77: 304–17.
57. Edbauer D, Winkler E, Regula JT, Pesold B, Steiner H, Haass C. Reconstitution of γ-secretase activity. Nat Cell Biol 2003; 5: 486–8.
58. Iwatsubo T. The γ-secretase complex: machinery for intramembrane proteolysis. Curr Opin Neurobiol 2004; 14: 379–83.
59. Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, Selkoe DJ. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. Nature 1999; 398: 513–7.
60. Ahn K, Shelton CC, Tian Y, Zhang X, Gilchrist ML, Sisodia SS, Li YM. Activation and intrinsic γ-secretase activity of presenilin1. Proc Natl Acad Sci USA 2010; 107: 21435–40.
61. Lessard CB, Wagner SL, Koo EH. And four equals one: presenilin takes the γ-secretase role by itself. Proc Natl Acad Sci USA 2010; 107: 21236–7.
62. Lichtenthaler SF, Haass C, Steiner H. Regulated intramembrane proteolysis-lessons from amyloid precursor protein processing. J Neurochem 2011; 117: 779–96.
63. Sastre M, Steiner H, Fuchs K, Capell A, Multhaup G, Condron MM, Teplow DB, Haass C. Presenilin-dependent γ-secretase processing of β-amyloid precursor protein at a site corresponding to the S3 cleavage of Notch. EMBO Rep 2001; 2: 835–41.
64. Weidemann A, Eggert S, Reinhard FB, Vogel M, Paliga K, Baier G, Masters CL, Beyreuther K, Evin G. A novel epsilon-cleavage within the transmembrane domain of the Alzheimer amyloid precursor protein demonstrates homology with Notch processing. Biochemistry 2002; 41: 2825–35.
65. Zhao G, Cui MZ, Mao G, Dong Y, Tan J, Sun L, Xu X. Gamma-cleavage is dependent on zeta-cleavage during the proteolytic processing of amyloid precursor protein within its transmembrane domain. J Biol Chem 2005; 280: 37689–97.
66. Qi-Takahara Y, Morishima-Kawashima M, Tanimura Y, Dolios G, Hirotani N, Horikoshi Y, Kametani F, Maeda M, Saido TC, Wang R, Ihara Y. Longer forms of amyloid β protein: implications for the mechanism of intramembrane cleavage by γ-secretage. J Neurosci 2005; 25: 436–45.
67. Kakuda N, Funamoto S, Yagishita S, Takami M, Osawa S, Dohmae N, Ihara Y. Equimolar production of amyloid β-protein and APP intracellular domain from β-carboxyl terminal fragment by γ-secretase. J Biol Chem 2006; 281: 14776–86.
68. Muller T, Meyer HE, Egensperger R, Marcus K. The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease. Prog Neurobiol 2008; 85: 393–406.
69. Blobel CP. ADAMs: key components in EGFR signaling and development. Nat Rev Mol Cell Biol 2005; 6: 32–43.
70. Liu PCC, Liu X, Li Y, Covington M, Wynn R, Huber R, Hillman M, Yang G, Ellis D, Marando C, Katiyar K, Bradley J, Abremski K, Stow M, Rupar M, Zhuo J, Li YL, Li Q, Burns D, Xu M, Zhang C, Qian DQ, He C, Sharief V, Weng L, Agrios C, Shi E, Metcalf B, Newton R, Friedman S, Yao W, Scherle P, Hollis G, Burn T. Identification of ADAM10 as a major source of ectodomain sheddase activity in HER2 overexpressing breast cancercells. Cancer Biol Ther 2006; 5: 657–64.
71. Bozkulak EC, Weinmaster G. Selective use of ADAM10 and ADAM17 in activation of Notch1 signaling. Mol Cell Biol 2009; 29: 5679–95.
72. Klaver DW, Wilce MCJ, Cui H, Hung AC, Gasperini R, Foa L., Small DH. Is BACE1 a suitable therapeutic target for the treatment of Alzheimer's disease? Current strategy and future direction. Biol Chem 2010; 391: 849–59.
73. Fleck D, Van Bebber F, Colombo A, Galante C, Schwenk BM, Rabe L, Hampel H, Novak B, Kremmer E, Tahirovic S, Edbauer D, Lichtenthaler SF, Schmid B, Willem M, Haass C. Dual cleavage of neuregulin1 type III by BACE1 and ADAM17 liberates its EGF-like domain and allows paracrine signaling. J Neurosci 2013; 33: 7856–69.
74. Wolfe S, Kopan R. Intramembrane proteolysis: theme and variations. Science 2004; 305: 1119–23.
75. De Strooper, Annaert W, Cupers P, Saftig P, Craessaerts K, Mumm JS, Schroeter EH, Schrijvers V, Wolfe MS, Ray WJ, Goate A, Kopan R. A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain. Nature 1999; 398: 518–22.
76. Struhl G, Greenwald I. Presenilin is required for activity and nuclear access of Notch in Drosophila. Nature 1999; 398: 522–5.
77. Selkoe D, Kopan R. Notch and presenilin: regulated intramembrane proteolysis links development and degeneration. Annu Rev Neurosci 2003; 26: 565–97.
78. Small DH, Clarris HL, Williamson TG, Reed G, Key B, Mok SS, Beyreuther K, Masters CL, Nurcombe V. Neurite-outgrowth regulating functions of the amyloid protein precursor of Alzheimer's disease. J Alzheimers Dis 1999; 1: 275–85.
79. Palmert MR, Usiak M, Mayeux R, Raskind M, Tourtellotte WW, Younkin SG. Soluble derivatives of the β amyloid protein precursor in cerebrospinal fluid: alterations in normal aging and in Alzheimer's disease. Neurology 1990; 40: 1028–34.
80. Hung AY, Selkoe DJ. Selective ectodomain phosphorylation and regulated cleavage of β-amyloid precursor protein. EMBO J 1994; 13: 534–42.
81. Ando K, Oishi M, Takeda S, Lijima KI, Isohara T, Nairn AC, Kirino Y, Greengard P, Suzuki T. Role of phosphorylation of Alzheimer's amyloid precursor protein during neuronal differentiation. J Neurosci 1999; 19: 4421–7.
82. Muresan Z, Muresan V. c-Jun NH2-terminal kinase-interacting protein-3 facilitates phosphorylation and controls localization of amyloid-β precursor protein. J Neurosci 2005; 25: 3741–51.
83. Murezan Z, Murezan V. Coordinated transport of phosphorylated amyloid-β precursor protein and c-Jun NH2-terminal kinase-interacting protein-I. J Cell Biol 2005; 171: 615–25.

84. Lee MS, Kao SC, Lemere CA, Xia W, Tseng HC, Zhou Y, Neve R, Ahlijania MK, Tsai LH. APP processing is regulated by cytoplasmic phosphorylation. J Cell Biol 2003; 163: 83–95.
85. Pastorino L, Sun A, Lu PJ, Zhou XZ, Balastik M, Finn G, Wulf G, Lim J, Li SH, Li X, Xia W, Nicholson LK, Lu KP. The proyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-β production. Nature 2006; 440: 528–34.
86. Ando K, Lijima KI, Elliott JI, Kirino Y, Suzuki T. Phosphorylation-dependent regulation of the interaction of amyloid precursor protein with Fe65 affects the production of β-amyloid. J Biol Chem 2001; 276: 40353–61.
87. Orcholski ME, Zhang Q, Bredesen DE. Signaling via amyloid precursor-like proteins APLP1 and APLP2. J Alzheimers Dis 2011; 23: 689–99.
88. Zheng H, Koo EH. The amyloid precursor protein: beyond amyloid. Mol Neurodegener 2006; 1: 1–12.
89. Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M. Alzheimer's disease: clinical trials and drug development. Lancet Neurol 2010, 9: 702–16.
90. Niemitz E. TREM2 and Alzheimer's disease. Nat Genet 2013; 45: 11.
91. Campion D, Dumanchin C, Hannequin D, Dubois B, Belliard S, Puel M, Thomas-Anterion C, Michon A, Martin C, Charbonnier F, Raux G, Camuzat A, Penet C, Mesnage V, Martinet M, Clerget-Darpoux F, Brice A, Fregourg T. Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. Am J Hum Genet 1999; 65: 664–70.
92. Lambert JC, Amouyel P. Genetic heterogeneity of Alzheimer's disease: complexity and advances. Psychoneuroendocrinology 2007; 32 (Suppl. 1): S62–70.
93. Mayeux R, Stem Y, Spanton S. Heterogeneity in dementia of the Alzheimer type: evidence of subgroups. Neurology 1985; 35: 453–61.
94. Gold G, Blouin JL, Herrmann FR, Michon A, Mulligan R, Sail GD, Bouras C, Giannakopoulos P, Antonarakis SE. Specific BACE1 genotypes provide additional risk for late-onset Alzheimer disease in APOE epsilon 4 carriers. Am J Med Genet B Neuropsychiatr Genet 2003; 119B: 44–7.
95. Dupont C, Armant DR, Brenner CA. Epigenetics: definition, mechanisms and clinical perspective. Semin Reprod Med. 2009; 27: 351–7.
96. Griffith JS, Mahler HR. DNA ticketing theory of memory. Nature 1969; 223: 580–2.
97. Kouzarides T. Chromatin modifications and their function. Cell 2007; 128: 693–705.
98. Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000; 403: 41–5.
99. Caceres JF, Kornblihtt AR. Alternative splicing: multiple control mechanisms and involvement in human disease. Trends Genet 2002; 18: 186–93.
100. Theuns J, Brouwers N, Engelborghs S, Sleegers K, Bogaerts V, Corsmit E, de Pooter T, van Duijn CM, de Deyn PP, van Broeckhoven C. Promoter mutations that increase amyloid precursor protein expression are associated with Alzheimer disease. Am J Hum Genet 2006; 78: 936–46.
101. Costa FF. Non-coding RNAs, epigenetics and complexity. Gene 2008; 410: 9–17.
102. Champagne FA. Epigenetics and developmental plasticity across species. Develop Psychobiol 2013; 55: 33–41.
103. Jones PA, Laird PW. Cancer epigenetics comes of age. Nat Genet 1999; 21: 163–7.
104. Meloni I, Bruttini M, Longo I, Mari F, Rizzolio F, D'Adamo P, Denvriendt K, Fryns JP, Tonolio D, Renieri A. A mutation in the Rett syndrome gene, MECP2, causes X-linked mental retardation and progressive spasticity in males. Am J Hum Genet 2000; 67: 982–5.
105. Tahiliani M, Mei P, Fang R, Leonor T, Rutenberg M, Shimizu F, Li J, Rao A, Shi Y. The histone H3K4 demethylase SMCX links REST target genes to X-linked mental retardation. Nature 2007; 447: 601–5.
106. Adegbola A, Gao H, Sommer S, Browning M. A novel mutation in JARID1C/SMCX in a patient with autism spectrum disorder (ASD). Am J Med Genet A 2008; 146A: 505–11.
107. Chouliaras L, Rutten BPF, Kenis G, Peerbooms O, Visser PJ, Verhey F, van Os J, Steinsbuch HWM, van den Hove DLA. Epigenetic regulation in the pathophysiology of Alzheimer's disease. Prog Neurobiol 2010; 90: 498–510.
108. Nguyen KV. Epigenetic regulation in amyloid precursor protein and the Lesch-Nyhan syndrome. Biochem Biophys Res Commun 2014; 446: 1091–5.
109. Lesch M, Nyhan WL. A familial disorder of uric acid metabolism and central nervous system function. Am J Med 1964; 36: 561–70.
110. Patel PI, Framson PE, Caskey CT, Chinault AC. Fine structure of the human hypoxanthine phosphoribosyl transferase gene. Mol Cell Biol 1986; 6: 393–403.
111. Fu R, Ceballos-Picot I, Torres RJ, Larovere LE, Yamada Y, Nguyen KV, Hegde M, Visser JE, Schretlen DJ, Nyhan WL, Puig JG, O'Neill PJ, Jinnah HA for the Lesch-Nyhan Disease International Study Group. Genotype-phenotype correlations in neurogenetics: Lesch-Nyhan disease as a model disorder. Brain 2014; 137: 1282–303.
112. Visser JE, Baer PR, Jinnah HA. Lesch-Nyhan disease and the basal ganglia. Brain Res Rev 2000; 32: 449–75.
113. Ceballos-Picot I, Mockel L, Potier MC, Dauphinot L, Shirley TL, Ibad RT, Fuchs J, Jinnah HA. Hypoxanthine-guanine phosphoribosyltransferase regulates early developmental programming of dopamine neurons: implications for Lesch-Nyhan disease pathogenesis. Hum Mol Genet 2009; 18: 2317–27.
114. Stacey NC, Ma MHY, Duley JA, Connolly GP. Abnormalities in cellular adhesion of neuroblastoma and fibroblast models of Lesch-Nyhan syndrome. Neuroscience 2000; 98: 397–401.
115. Harris JC, Lee RR, Jinnah HA, Wong DF, Yaster M, Bryan RN. Craniocerebral magnetic resonance imaging measurement and findings in Lesch-Nyhan syndrome. Arch Neurol 1998; 55: 547–53.
116. Schretlen DJ, Varvaris M, Ho TE, Vannrsdall TD, Gordon B, Harris JC, Jinnah HA. Regional brain volume abnormalities in Lesch-Nyhan disease and its variants: a cross-sectional study. Lancet Neurol 2013; 12: 1151–8.
117. Schretlen DJ, Varvaris M, Tracy D, Vannorsdall TD, Gordon B, James C, Harris JC, Jinnah HA. Brain white matter volume abnormalities in Lesch-Nyhan disease and its variants. Neurology 2014 (in press, Epub ahead of print, December 10, 2014, doi: 10.1212/WNL.0000000000001128).
118. Kang TH, Guibinga, Friedmann T. HPRT deficiency coordinately dysregulates canonical Wnt and presenilin-1 siggnaling: a neurodevelopmental regulatory role for a housekeeping gene? PloS One 6 (e16572) 2011; 1–11.
119. Kang TH, Park Y, Bader JS, Friedmann T. The housekeeping gene hypoxanthine-guanine phosphoribosyltransferase (HPRT)

regulates multiple developmental and metabolic pathways of murine embryonic stem cell neuronal differentiation. PloS One 8 (e74967) 2013; 1–15.
120. Moore JH. The ubiquitous nature of epistasis in determining susceptibility to common human diseases. Hum Hered 2003; 56: 73–82.
121. Combarrros O, Borja MC, Smith AD, Lehmann DJ. Epistasis in sporadic Alzheimer's disease. Neurobiol Aging 2009; 30: 1333–49.
122. Nguyen KV, Naviaux RK, Paik KK, Nakayama T, Nyhan WL. Lesch-Nyhan variant syndrome: real-time RT-PCR for mRNA quantification in variable presentation in three affected family members. Nucleosides Nucleotides Nucleic Acids 2012; 31: 616–29.
123. Ceballos-Picot I, Auge F, Fu R, Bandini AO, Cahu J, Chabrol B, Aral B, de Martinvill B, Lecain JP, Jinnah HA. Phenotype variation among seven members of one family with deficiency of hypoxanthine-guanine phosphoribosyltransferase. Mol Genet Metab 2013; 110: 268–74.
124. Wenli G, Feng Z, Lupski JR. Mechanisms for human genomic rearrangements. PathoGenet 2008; 1: 4.
125. Lupski JR, Strankiewicz P. Genomic disorders: molecular mechanisms for rearrangements and conveyd phenotypes. PLoS Genet 2005; 1: e49.
126. Lupski JR. Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. Trends Genet 1998; 14: 417–22.
127. Lupski JR. Genomic disorders ten years on. Genome Med 2009; 1: 42.1–42.11.
128. Sebat J, Lakshmi B, Malhotra D, Troge J, Lee-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, Leotta A, Pai D, Zhang R, Lee YH, Hicks J, Spence SJ, Lee AT, Puura K, Lehtimaki T, Ledbetter D, Gregersen PK, Bregman J, Scutcliffe JS, Jobanputra V, Chung W, Warburton D, King MC, Skuse D, Geschwind DH, Gilliam TC, Ye K, Wigler M. Strong association of de novo copy number mutations with autism. Science 2007; 316: 445–9.
129. Lupski JR. Schizophrenia: incriminating genomic evidence. Nature 2008; 455: 178–9.
130. Stankiewicz P, Beaudet AL. Use of od array CGH in the evaluation of dysmorphology, malformations, developmental delay, and idiopathic mental retardation. Curr Opin Genet Dev 2007; 17: 182–92.
131. Chen JM, Chuzhanova N, Stenson PD, Ferec C, Cooper DN. Complex gene rearrangements caused by serial replication slippage. Hum Mutat 2005; 26: 125–34.
132. Lee JA, Lupski JR. Genomic rearrangements and gene copy-number alterations as a cause of nervous system disorders. Neuron 2006; 52: 103–21.
133. Thomas P, Frederick WA. Cis-regulatory elements and epigenetic changes control genomic rearrangements of the IgH locus. Adv. Immunol. 2008; 99: 1–32.
134. Guttmacher AE, Collins FS. Alzheimer's disease and Parkinson's disease. N Engl J Med 2003; 384: 1356–64.
135. Ertekin-Taner N. Genetics of Alzheimer disease in the pre-and post-GWAS era. Alzheimer's Res Ther 2010; 2: 3.
136. Joseph J, Shukitt-Hale B, Denisova A, Martin A, Perry G, Smith MA. Copernicus revisited: amyloid β in Alzheimer's disease. Neurobiol Aging 2001; 22: 131–46.
137. Bishop GM, Robinson SR. The amyloid hypothesis: let sleeping dogmas lie? Neurobiol Aging 2002; 23: 1101–5.
138. Reitz C. Alzheimer's disease and the amyloid cascade hypothesis: a critical review. Internat J Alzheimer's Dis 2012; Article ID: 369808, 11 pages, doi: 10.1155/2012/369808.
139. Graeber MB, Kosel S, Egensperger R, Banati RB, Muller U, Bise K, Hoff P, Moller HJ, Fujisawa K, Mehraein P. Rediscovery of the case described by Alois Alzheimer in 1911: historical, histological and molecular genetic analysis. Neurogenetics 1997; 1: 73–80.
140. Muller U, Winter P, Graeber MB. Alois Alzheimer's case, Auguste D., did not carry the N141I mutation in PSEN2 characteristic of Alzheimer disease in Volga Germans. Arch Neurol 2011; 68: 1210–1.
141. Alagiakrishman K, Gill SS, Fagarasanu A. Genetics and epigenetics of Alzheimer's disease. Postgrad Med J 2012; 88: 522–9.
142. Bentahir M, Nyabi O, Verhamme J, Tolia A, Horre K, Wiltfang J, Esselmann H, De Strooper B. Presenilin clinical mutations can affect γ-secretase activity by different mechanisms. J Neurochem 2006; 96: 732–42.
143. Sokol DK, Chen D, Farlow MR, Dunn DW, Maloney B, Zimmer JA, Lahiri DK. High levels of Alzheimer β-amyloid precursor protein (APP) in children with severely autistic behavior and aggression. J Child Neurol 2006; 21: 444–9.
144. Ray B, Long JM, Sokol DK, Lahiri DK. Increased secreted amyloid precursor protein-α (sAPPα) in severe autism: proposal of a specific, anabolic pathway and putative biomarker. PLoS ONE 2011; 6: e20405.
145. Sokol DK, Maloney B, Long JM, Ray B, Lahiri DK. Autism, Alzheimer disease, and fragile X. APP, FMRP, and mGluR5 are molecular links. Neurology 2011, 76: 1344–52.
146. Lahiri DK, Sokol DK, Erickson C, Ray B, Ho CY, Maloney B. Autism as early neurodevelopmental disorder: evidence for an sAPPα-mediated anabolic pathway. Front Cell Neurosci 2013; 7: 1–13.
147. Bennett CF, Swayze EE. RNA targeting therapeutics: molecular mechanisms of antisense oligonucleotides as a therapeutic platform. Annu Rev Pharmacol Toxicol 2010; 50: 259–93.
148. Saonese JA. Antisense therapy, a magic bullet for the treatment of various diseases: present and future prospects. J Med Genet Genomics 2011; 3: 77–83.
149. Watts JK, Corey DR. Silencing disease genes in the laboratory and the clinic. J Pathol 2012; 226: 365–79.

Supplemental Material: The online version of this article (DOI: 10.1515/bmc-2014-0041) offers supplementary material, available to authorized users.

Bionote



Khue Vu Nguyen
Department of Medicine, Biochemical Genetics and Metabolism, The Mitochondrial and Metabolic Disease Center, School of Medicine, University of California, San Diego, Building CTF, Room C-103, 214 Dickinson Street, San Diego, CA 92103, USA; and Department of Pediatrics, University of California, San Diego, School of Medicine, San Diego, La Jolla, CA 92093, USA
kvn006@ucsd.edu

Dr. Khue Vu Nguyen (born in Ha Noi, Vietnam) is currently Full Project Scientist in the Departments of Medicine and Pediatrics, University of California, San Diego, School of Medicine, San Diego, CA, USA. He has studied at the University Louis Pasteur, Strasbourg, France: where he received his BS in Biochemistry, his MS in Molecular Biology, his PhD in Macromolecular Physical Chemistry, and his PhD in Physical Sciences (Doctorat d'Etat es Sciences Physiques). He has worked as a scientist in different French and US research institutions and companies. He is author and co-author of numerous publications and holder of many patents. Dr. Nguyen is listed in Who's Who in the World, Who's Who in America, etc. He is a member of academic organizations such as the American Society for Microbiology and the American Chemical Society. He is an invited reviewer of different scientific journals such as Molecular Genetics and Metabolism and AGE: Journal of the American Aging Association. He is also an editorial board member of different scientific journals such as the International Journal of Medicine and Molecular Medicine and Open Journal of Endocrine and Metabolic Diseases. He is Scientist Reviewer for the Metabolic Disease Two (MB-2) of the Peer Review Medical Research Program (PRMRP) for the U.S. Department of Defense Congressionally Directed Medical Research Programs (CDMRP). His current research includes genetic diseases, neurodevelopmental and neurodegenerative disorders and cancer.