Mutational burden of giant synaptic genes may be the cause of Alzheimer's disease

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Abstract

All of the drug trials of the Alzheimer's disease (AD) have failed to slow progression of dementia in phase III studies, and the most effective therapeutic approach still remains controversial due to our incomplete understanding of AD pathophysiology. Amyloid beta (Aβ) and its cascade have been the primary focus of drug design efforts for more than a decade. However, mounting evidence indicates that mechanisms of AD etiopathogenesis are probably more complex than the previous reductionist models.

Several genome-wide association studies (GWAS) have recently shed light on dark aspects of AD from a hypothesis-free point of view. While the newly-identified AD risk genes rather raise more questions than they answer in deciphering the amyloid cascade, as a potentially overlooked finding, many of them code for receptors and transducers of cell adhesion signaling cascades. Remarkably, the hallmark genetic factors of AD, including the amyloid precursor protein (APP), presenilins (PSEN) and APOE also take part in highly similar pathways of cell adhesion regulation and coordinate contact-guidance of neuronal growth cones in brain development, albeit these Aβ-independent roles remain highly underexplored.

Here, we have revisited function of 27 AD risk genes in pathways of normal cell physiology. Our review clearly shows that a disrupted cell adhesion signaling nexus, rather than a protein aggregation process, is the central point of convergence in the unbiased genetic risk factors of AD. To further elucidate a potential relationship between aging and pathways of cell adhesion, we have conducted an exploratory bioinformatics analysis which revealed that *cell adhesion* is the most representative ontology of human genes larger than 500kb ($p=8.0\times10^{-13}$), and these extremely large genes are mostly expressed in brain ($p=2.1\times10^{-17}$) and selectively take part in synaptic composition ($p=2.4\times10^{-14}$). As possible driving forces of brain evolution, large genes may coordinate complex wiring of synaptic circuits in neurodevelopment, and we suggest that they may also be vulnerable to the impact of somatic mutations in aging due to their exceptional sizes which will be assessed by statistical models. An exemplar of this notion is the giant APOE receptor Lrp1b which is one of the most frequently deleted genes in various cancers and also represents the only brain-specific lipoprotein receptor. Our model, the *large gene instability hypothesis*, highlights alternate strategies for AD prevention, biomarker discovery and therapeutic design based on targeting genomic instability and synaptic adhesion.

Keywords: Alzheimer's disease; dementia pathogenesis; cell adhesion; integrin; focal adhesion kinase; DNA damage

Abbreviations: Alzheimer's disease (AD), Amyloid precursor protein (APP), Genome-wide association study (GWAS), Focal adhesion kinase (FAK), Postsynaptic density (PSD), Presenilin (PSEN), Src family kinase (SFK)

Introduction

More than a century has passed since the first report of a presenile dementia case by Alois Alzheimer[1], and current molecular understanding of AD mostly borrows from identification of the Aβ peptide as the main constituent of senile plaques and subsequent discovery of APP and PSEN mutations in rare cases of familial AD[2,3]. These observations were compiled to the amyloid cascade hypothesis in the pre-genomic era, a theory which implicates Aβ species, proteinous aggregates and neurofibrillary tangles as the driving force of dementia with broad influence as the central model of AD etiopathogenesis[4].

Nevertheless, due to various methodological difficulties, Aβ species has hardly been validated as a causal force of neurodegeneration in AD patients. Despite receiving support from preclinical studies, manipulating the pathways of Aβ generation and clearance has also yielded disappointing results in several clinical trials so far[5]. While a handful of clinical failures do not necessarily disprove a theory *per se*, overemphasis on a single disease model is a dangerous gamble and could be one of the many explanations for the lack of progress in AD therapeutic design[6]. Accuracy of the amyloid cascade hypothesis is a topic of ongoing debate, and it goes without saying that this theory may be rejected in future[7-12], a critical possibility warranting development of alternate disease models for interpreting exploratory evidence including recent high-throughput genomic findings.

In contrast to the classical hallmarks of AD including senile plaques and neurofibrillary tangles that are still of questionable etiological significance[13], genetic risk factors temporally precede earliest stages of brain development, aging, and neurodegeneration, and are expected to inform on causal disease pathways. The genetic architecture of common sporadic AD is highly complex, and a number of susceptibility loci have been identified by genome-wide association studies (GWAS) in large elderly populations. These novel genetic factors provide unbiased insight into molecular and cellular mechanisms of AD[14-19] but their mechanistic interpretation has been under powerful influence of the amyloid cascade theory so far.

This report servers to provide an evidence-based framework for compiling pathways of AD predisposition from an Aβ-independent point of view. The rest of this manuscript is organized as follows; in the first section, we aim to revisit the role of AD risk genes in pathways of normal cell physiology. We show that 27 disease-related genes strongly converge to common pathways of cell-extracellular adhesion signaling and brain circuit development. In the second section, we will try to explain interaction of aging as the strongest risk factor of senile dementias with the genetic landscape of AD. Finally, several testable predictions are provided for assessment of our new disease model.

1.1 The APP family proteins are highly-conserved cell adhesion molecules

Derailed catabolism of the APP protein and generation of an aggregation-prone Aβ species abstract a significant proportion of molecular investigations in AD, leading to efforts to block this cascade by means of Aβ immunotherapies or design of secretase inhibitors[5]. In contrast,

three decades after successful cloning of the APP gene[20] the physiological role of its protein product remains less investigated and may be the real key to understanding disease mechanisms.

APP codes for a transmembrane protein and is highly expressed in the developing brain at the site of neuronal growth cones, structures that form motile tips of outgrowing axons and dendrites[21]. The Aβ peptide enhances interaction of developing neurites with extracellular adhesion molecules and promotes outgrowth of neuronal projections[22,23]. The full-length and membrane-tethered form of APP also interacts with extracellular laminin, heparan sulfate, fibronectin and collagen[24-26], molecules which form the backbone of extra-cellular matrix and moderate contact-guidance of growth cones in synaptic circuit formation.

Interaction of APP with heparan sulfate[27] and laminin[24] stimulates assembly of hippocampal connections and promote neurite outgrowth[28]. In the other hand, antisense-downregulation of APP inhibits extension of axons and dendrites[29]. APP demonstrates a dose effect in growth cone guidance[30] and its increased dosage in Down syndrome results in emergence of faster advancing growth cones with promoted adhesive properties and larger sizes[31]. In contrast, knockdown of the APP gene in zebrafish disrupts outgrowth of developing neurites[32]. Intriguingly, although wild-type human APP can rescue this defective phenotype, the mutated APP of familial AD fails to substitute for normal function of animal gene[32].

Several cellular pathways are speculated to mediate the neurite-promoting effects of APP in neurodevelopment. The netrin pathway of neurite guidance incorporates APP as a co-receptor[33], and inactivation of APP disrupts netrin signaling and diminishes axonal outgrowth[34]. APP also binds reelin, which is a large extracellular adhesion molecule for guidance and migration of neurons[35]. In this context, interaction of reelin with APP promotes outgrowth of hippocampal neurites[35]. Functional interaction of APP and reelin requires presence of a third cell adhesion molecule, α3β1-integrin, as well[35]. Integrins are the main component of focal adhesions and known to co-localize with the APP protein[36,37] at dynamic neuronal adhesion sites[38]. Intriguingly, integrin modulates neurite outgrowth by interacting with APP[39]. Similarly, integrin also acts as an accessory reelin receptor for cell adhesion regulation and neuronal migration[40-42]. Therefore, APP, integrin, reelin (and its counterpart APOE receptors) may take part in surface adhesion ligand/receptor complexes for transduction of coherent signals.

In addition to growth cone navigating, APP also moderates spatial migration of neurons in neurodevelopment[43]. Triple-knockout of the APP family genes results in neuronal migration defects similar to human lissencephaly[44] and two candidate ligands for APP, including pancortin and lingo1, orchestrate migration of neural precursor cells[45-47]. From a cellular and molecular point of view, pathways of growth cone navigation and cell migration are highly similar, as both of these events rely on specialized membrane protrusions, namely filopodia and lamellipodia for cell reshaping and anchorage. These plastic cell organizations sense the directional gradients of extracellular contact-guidance cues by means of surface adhesion receptors including integrins. Intracellularly, filopodial adhesion receptors affect rearrangement of the actin cytoskeleton for changing cell polarity and recycling focal adhesion turnover towards the protruding end and powering cell movement[48].

Mounting evidence indicates that the cytoskeletal system is an important point of convergence in signaling through the APP protein. Transmembrane APP is selectively localized to the cytoskeletal-rich regions of neuronal growth cones at dynamic adhesion sites[38,49], and the APP intracellular domain (AICD) which is released after γ-secretase-mediated cleavage affects rearrangement of the cellular actin cytoskeleton[50]. The AICD cleavage fragment of APP interacts with a number of intracellular signal transducers, including Fe65, Tip60, KAI1, DISC1, Dab1, X11, and Grb2 that have been identified to date[51-53]. Intriguingly, all of these transducers influence pathways of cytoskeletal rearrangement and affect cell movement:

- Fe65 and Tip60 affect the cytoskeletal system and moderate cancer cell migration[54].
- KAI1 suppresses cancer cell migration by affecting cytoskeletal assembly[55,56].
- DISC1 coordinates remodeling of the actin cytoskeleton in migrating neurons and growth cone-like protrusions[57]. This protein rescues neuronal migration defects caused by loss of APP[51].
- Dab1 is a mandatory adaptor of the APOE receptors in the reelin pathway and controls cytoskeletal remodeling in neuronal migration[58].
- X11 is a recently-discovered modulator of the reelin pathway and affects cell movement[59].
- Grb2 is an adaptor molecule that links various receptors including integrin with intracellular pathways of cytoskeletal plasticity and regulates cancer cell migration[60,61].

In accordance with the common function of these putative signal transducers, The APP family genes similarly affect migration and invasion of various cancer cells by affecting the cytoskeletal pathway[62,63]. Interestingly, the key cytoskeletal regulator Rac1 controls expression of the APP gene by a feedback-like mechanism in primary hippocampal neurons[64].

Since the early stages of nervous system evolution, the APP paralogue of Drosophila (APPL) has promoted neuronal migration[65]. Phylogenetic evolution of the APP family genes reveals that cell adhesion is the most consistent biological function of this family[66]. From an evolutionary point of view, the cytoplasmic tail of APP is probably of utmost importance, as it comprises a super-conserved NPxY amino acid motif in the form of $_{682}$YE**NP**T**Y**$_{687}$ that has remained unchanged from roundworms to humans for more than 900 million years of evolution[67]. This consensus motif is known to mediate endocytic sorting of receptors and, perhaps more importantly, their interaction with tyrosine-phosphorylated intracellular signal transducers[68]. Two intracellular adaptors of APP with established signaling roles, including Dab1 and Fe65, interact with this APP motif in a phosphorylation-dependent manner[69,70]. The $_{682}$Tyr residue of this APP motif undergoes phosphorylation and is essential to induction of synaptogenesis[71]. Of note, the consensus NPxY motif is also present in the cytoplasmic tail of all APOE receptors and activates the mandatory Dab1 adaptor of reelin pathway[72].

In addition to playing physiological roles in neurodevelopment, the APP protein is evidenced to maintain its function in mature neurons. Mouse hippocampal neurons express the APP protein under physiological conditions[73], and APP is present in close association with NMDA glutamate receptors that are central to memory-formation. In this context, APP maintains NMDA receptors at postsynaptic membrane and promotes neurotransmission[74,75]. Through its conserved NPxY

178 motif, APP also interacts with the postsynaptic scaffold protein AIDA-1[76], which is a protein for
179 regulating hippocampal synaptic transmission and palsticity[77]. Loss of the APP family genes
180 disrupts synaptic function[78], memory formation[79], and causes an aging-related synaptic loss in
181 mice[80,81]. Further supporting physiological roles in synaptic adhesion, the three APP family
182 members (APP, APLP1, APLP2) form trans-synaptic adhesive dimers[82], and cleavage of the
183 APP protein changes synaptic adhesion and assembly[83]. Lastly, APP mutations are shown to
184 disrupt this regulatory effect[84]. An exhaustive review of the APP protein in pathways of normal
185 cell physiology is beyond the scope of this manuscript and the interested reader is referred to
186 recent publications[85-87].

187 1.2 The γ-secretase complex is a membrane-tethered enzyme for signaling of cell
188 adhesion receptors

189 PSEN1 and PSEN2 genes code for catalytic subunits of the transmembrane γ-secretase enzyme
190 and various mutations in these genes cause autosomal-dominant AD. Since cleavage of the APP
191 protein by γ-secretase is a mandatory step for Aβ generation, accelerated catabolism of APP in
192 the amyloidogenic pathway is considered the mechanism of AD development in individuals with
193 mutated PSEN genes, a hypothesis which has been extrapolated to common sporadic AD as well.

194 Unexpectedly, PSEN mutations of familial AD were recently found to cause an almost complete
195 loss of γ-secretase function[88] and reduce generation of the putatively-neurotoxic $A\beta_{40}$, $A\beta_{42}$ and
196 $A\beta_{43}$ species occasionally to undetectable levels[89,90]. In further contradiction, knock-in mice
197 harboring the mutated PSEN1 gene of familial AD are phenotypically similar to knockout strains
198 which lack γ-secretase function, and both of these strains demonstrate impaired hippocampal
199 plasticity[91]. This line of evidence suggests a loss-of-function mechanism for PSEN mutations of
200 familial AD, and potentially explain the paradoxical worsening of cognitive function and
201 accelerated brain atrophy in γ-secretase inhibitor trials of AD[92,93].

202 In contrast to the narrow focus on derailed pathways of APP catabolism, unbiased proteomic
203 profiling has revealed that the γ-secretase complex has a broad spectrum of substrate specificity
204 for cell surface receptors with signaling roles[94,95]. For instance, the γ-secretase enzyme cleaves
205 the APOE/reelin receptors[96], as well as DSG2, TREM2 and ephrin receptors which are all coded
206 by AD risk genes[94,97,98]. A candidate gene of familial AD, Notch3[99], is mandatorily cleaved by γ-
207 secretase prior to signaling[100]. Loss of γ-secretase results in erroneous axonal pathfinding due to
208 derailed netrin signaling[101], and has also been shown to disrupt cell adhesion force generation[102].
209 While it is difficult to pinpoint a particular signaling path that mediates detrimental effects of γ-
210 secretase dysfunction in familial AD, it is tempting to speculate pathways of synaptic adhesion
211 and contact-guidance in its etiopathogenesis.

212 Recent nanoscale microscopy reveals that expression of the γ-secretase complex is selectively
213 enriched in postsynaptic compartments during normal synaptic maturation[103]. A
214 neurophysiological role for γ-secretase is further supported by observing that this enzyme
215 interacts with a number of synaptic adhesion molecules including δ-catenin and N-cadherin, as
216 well as glutamate receptors[103,104]. Cleavage activity of γ-secretase modulates synaptic

transmission and adhesive properties[104] and this neuromodulatory effect is disrupted by familial AD mutations[105].

1.3 The APOE/Lipoprotein receptor pathway coordinates contact-guidance of neurites

APOE4 is the strongest genetic risk factor of sporadic AD and explains ~6 percent of the risk of disease development[106]. In contrast, the only observed correlation of the APP locus with sporadic AD has been recently reported in a large Icelandic cohort, showing that a rare protective variant, A673T, explains less than 0.6 percent of the risk of sporadic AD[107]. This variant does not contribute to the risk of AD in the North American population[108], and its statistical significance ($p=4.8\times10^{-7}$) would not have survived a similarly-powered genome-wide scan. Curiously, mechanistic interpretation of the APOE4 risk isoform still mostly borrows from putative influences on APP catabolism and Aβ clearance, and normal function of the APOE protein has received less attention.

The APOE molecule moderates transport of lipoprotein particles in various organs by binding to the family of lipoprotein receptors. Although lipoprotein receptors aid in uptake and metabolism of lipid particles, they are not simple cargo transporters, and can activate a comprehensive nexus of intracellular second messengers with specialized signaling roles[109]. In this context, lipoprotein receptors including APOEr2 and VLDLr are well established regulators of brain development in reelin signaling. Activation of these two receptors by reelin triggers phosphorylation of the intracellular Dab1 adaptor. This pathway affects various aspects of cell physiology, among which cytoskeletal remodeling and neuronal migration are mainstay[110]. The reelin pathway guides extension of hippocampal neurites[111] and coordinates outgrowth of the perforant path which represents the major input to hippocampal formation[112].

The APOE molecule shares its lipoprotein receptors with reelin[113], and mounting evidence indicates that APOE undertakes a similar role in guiding outgrowth of developing neurites[113-117]. Moreover, the neurite promoting effect of APOE is isoform-dependent, with the APOE3 isoform being a more potent inducer of neuritic outgrowth than APOE4[115,117].

Intracellular transducers of the APOE molecule remain less investigated in neurons, but have been explored in other cells. In macrophages, APOE activates major transducers of the reelin pathway including Dab1 and PI3K[118]. In vascular pericytes, the APOE molecule affects rearrangement of the actin cytoskeleton and its knockdown deranges normal cell migration[119]. Similar to vascular cells, the APOE isoforms also affect the proteomic signature of cytoskeletal regulators in peripheral nerves[120]. Taken together, this body of evidence suggests that APOE may signal through a reelin-like pathway and influence cytoskeletal assembly, neurite outgrowth and cell movement.

In addition to the APOE risk locus and the reelin gene which is correlated with AD neuropathology in postmortem human brains[121], three novel AD susceptibility loci further implicate lipoprotein receptors and a reelin-like signaling pathway in this disease; F-spondin (Spon1) is correlated with rate of cognitive decline in AD and also modulates white matter

microstructure in healthy humans[122,123]. This gene codes for a reelin domain-containing cell adhesion molecule and its ortholog localizes to integrin adhesion sites in C. elegans[124]. F-spondin also binds APOEr2[125] and guides extension of hippocampal neurites[126]. Moreover, F-spondin interacts with the APP protein[127], and this interaction serves to activate signaling of the reelin adaptor Dab1 in ganglion cells[128]. Two other AD risk loci including Sorl1[129,130] and CLU respectively code for a lipoprotein receptor and a lipoprotein receptor ligand. Sorl1 regulates cell migration[130,131] and CLU activates reelin transducers including Dab1 and PI3K/Akt in neurons[132].

Perhaps unrelated to their roles in lipid metabolism, lipoprotein receptors take part in the architecture of postsynaptic structures by interacting with the major synaptic scaffold protein PSD95[133-135] as well as neurotransmitter receptors[133,135]. Expression of lipoprotein receptors affect synaptic density in hippocampal and cortical neurons[136], and their activation by reelin promotes synaptic plasticity[137-139]. Intriguingly, lipoprotein receptors share several intercellular transducers with the APP protein, including X11, Dab1, Fe65[136,140] and also control transcriptional activation of the APP gene in the cell nucleus[141].

1.4 AD susceptibility loci strongly converge to cell adhesion pathways

Familial AD which is caused by APP or PSEN mutations constitutes less than one percent of AD cases. In contrast, the true polygenic landscape of common sporadic AD has been partly uncovered by recent genome-wide association studies[14-19]. Remarkably, the majority of novel AD risk genes engage in pathways of cell adhesion, migration and contact-guidance:

- **DSG2** (Desmoglein-2, rs8093731) is a component of cell adhesion complexes. DSG2 gene product serves focal adhesion roles and regulates cytoskeletal assembly by interacting with β8-integrin in endothelial cells[142]. DSG2 also controls cell motility and its depletion affects migration of malignant melanoma cells[143].
- **EPHA1** (rs11771145) codes for a member of the ephrin-A receptor family that controls neurite adhesion and guidance. EPHA1 also moderates cell migration through integrin-linked kinase and the cytoskeletal remodeling pathway[144,145], and affects invasion and metastasis of colorectal cancer cells[146].
- **FRMD4A**[147] and **FERMT2** (Kindlin-2, rs17125944) code for two members of the FERM domain family linking integrin and focal adhesion kinase (FAK) with the intracellular actin cytoskeleton[148,149]. FERMT2 transduces cell adhesion signals and is engaged in malignant cell invasion[150].
- **GAB2** (rs2373115), one of the earliest AD susceptibility genes to be discovered by GWAS[14,151], encodes a scaffolding protein acting downstream to the integrin signaling pathway. GAB2 regulates adhesion and migration of hematopoietic cells[152] and also controls cytoskeletal remodeling for migration of malignant breast cancer cells[153].
- **CASS4** (Hepl, rs7274581) controls focal cell adhesion[154] and the CAS family members take part in axon guidance by interacting with integrin[155]. CASS4 also affects reorganization of the cytoskeleton and moderates cancer cell invasion[154,156].

- **CD2AP** (rs10948363) codes for an actin cytoskeleton binding protein[157]. CD2AP regulates focal adhesion of kidney podocytes at contact sites by linking membrane adhesion complexes with the intracellular actin cytoskeleton[158].
- **PTK2B** (Pyk2, rs28834970) is a focal adhesion signal transducer and affects the cellular cytoskeleton[159,160]. PTK2B controls integrin-dependent migration of T-cells[161] and promotes invasion of malignant glioma cells[162].
- **PICALM** (rs10792832) is a Clathrin adaptor protein and engages in membrane receptor trafficking[163]. Clathrin regulates endocytosis of synaptic vesicles and moderates trafficking of the glutamate receptors[164]. Unbiased gene-gene interaction analysis in AD has revealed that the PICALM locus interacts with DOCK1[165], which is an actin cytoskeleton regulator and affects cell movement[166].
- **INPP5D** (SHIP-1, rs35349669) is a key modulator of the PI3K pathway. This protein regulates platelet adhesion by modulating integrin signaling[167] and also coordinates movement of neutrophils in response to focal contact and adhesion[168].
- **NYAP1** (rs1476679) codes for a signal transducer of the PI3K pathway. NYAP1 acts downstream to Contactin5 synaptic adhesion molecule and controls cytoskeletal remodeling in neurite outgrowth[169]. Of note, Contactin5 binds amyloid precursor-like protein 1[170].
- **Amphysin II** (BIN1, rs6733839) codes for a protein that binds the cytoplasmic tail of integrin[171] and neuronal focal adhesion kinase[172] and is therefore probably involved in integrin-dependent cell adhesion. Moreover, Amphysin I which has a high level of sequence similarity (71%) with this gene product regulates outgrowth of hippocampal neurites[173] and links endocytosis mechanisms to pathways of actin cytoskeleton remodeling[174].
- **UNC5C**[175] (rs137875858) codes for a receptor of the netrin pathway of axon guidance[176]. In addition to the noted interaction of netrin1 with the APP protein, the netrin pathway also incorporates α3β1-integrin and Down Syndrome Cell Adhesion Molecule (DSCAM) in neuronal migration and neurite outgrowth, respectively[177,178].
- **TPBG**, a recently discovered AD risk gene[19], modulates cell adhesion and movement[179,180]. TPBG localizes at focal adhesion sites in kidney podocytes and affects formation of actin stress fibers for cell remodeling[181]. Deletion of TPBG disrupts cadherin-dependent cell adhesion and suppresses cell migration[182].
- **HBEGF**[19] (rs11168036) encodes a protein that promotes integrin-dependent cell adhesion[183]. HBEGF also regulates focal adhesion kinase and moderates cell migration by affecting the actin cytoskeleton[184].
- **USP6NL**[19] (RNTRE, rs7920721) modulates integrin signaling and controls focal adhesion turnover, thereby acting as a "brake" in cell migration[185].
- **TREM2** (rs75932628), a novel AD locus identified by next-generation sequecing[186], is known to interact with the plexin-A1 adhesion molecule[187] which is a receptor of axon guidance. The TREM and plexin-A1 interaction is suggested to moderate cell adhesion and movement through the cytoskeletal pathway[188]. The Plexin pathway also opposes integrin signal and inhibits cell movement[189].

- **TTC3**, a novel familial late-onset AD locus, maps to the Down syndrome critical region[190]. TTC3 modulates β1-integrin signaling in cancer cells[191] and its increased level affects assembly of the actin cytoskeleton and disrupts neurite extension[192].
- **PLCG2**[193] (rs72824905) encodes a phospholipase enzyme which is activated by integrin and moderates cell migration[194]. PLCG2 activation controls adhesion of leukocytes and takes place downstream to integrin signaling[195].
- **ABI3**[193] (rs616338) affects the cytoskeletal pathway and participates in formation of membrane protrusions for cell motility[196]. Its binding partner, the ABI3 binding protein, interacts with integrin at focal adhesion sites and suppresses malignant cell migration[197,198].

Taken together, the genetic architecture of AD strongly implicates various cell adhesion receptors which coordinate pathways of cytoskeletal plasticity and cell reshaping. Further aiding in formulation of a unified disease model, many of these gene products cross-talk with the integrin pathway, and this convergence spotlights the Aβ-independent roles of APP and γ-secretase in cell adhesion signaling and synapse formation.

2 The hypothesis

Our model builds on the unbiased genetic architecture of AD and puts the cell adhesion signaling pathway at the center of disease nexus. Various cell adhesion regulators including integrins coordinate cell migration, neurite elongation, and assembly of synaptic circuits in brain development, and also undertake pivotal roles in maintaining synaptic integrity and homeostasis after completion of brain development[199]. Specifically, cell adhesion molecules form a dense scaffold at the postsynaptic density (PSD) sites and connect neurotransmitter receptors and synaptic ion channels with the actin cytoskeleton as well as the extracellular matrix. In addition to such mechanical support, synaptic adhesion molecules also act as biochemical sensors for modulation of postsynaptic plasticity, dendritic spine reshaping, and recycling of transmitter receptors[200]. For instance, it has been shown that enhancing signaling of synaptic integrin by application of its agonist peptide modulates neurotransmission[201] in a dose-dependent manner[202]. In this context, integrin promotes budding of filopodia which serve to strengthen synaptic connections by cytoskeletal plasticity mechanisms[203], i.e. the same mechanism by which integrins control axonal adhesion and pathfinding during brain development[204]. Remarkably, many of the intracellular transducers recruited by focal adhesion cascades also act as molecular switches of synaptic plasticity, including various tyrosine kinases (SFK, PI3K and Akt) as well as the calcium signaling pathway[205].

The post-developmental roles of cell adhesion pathways in synaptic function and plasticity, which is not limited to integrins (Fig. 1), may enlighten pathways of AD from an Aβ-independent perspective. We propose that the heritable component of AD is defined by several genetic factors that coordinate robust adhesion and assembly of synaptic circuits in brain development. This genetic landscape also defines the level of synaptic adhesion, integrity and circuit resilience in the post-developmental period, and individuals with a vulnerable genetic

376 background may suffer disassembly of neural circuits due to loss of synaptic adhesion pathways
377 in late life.

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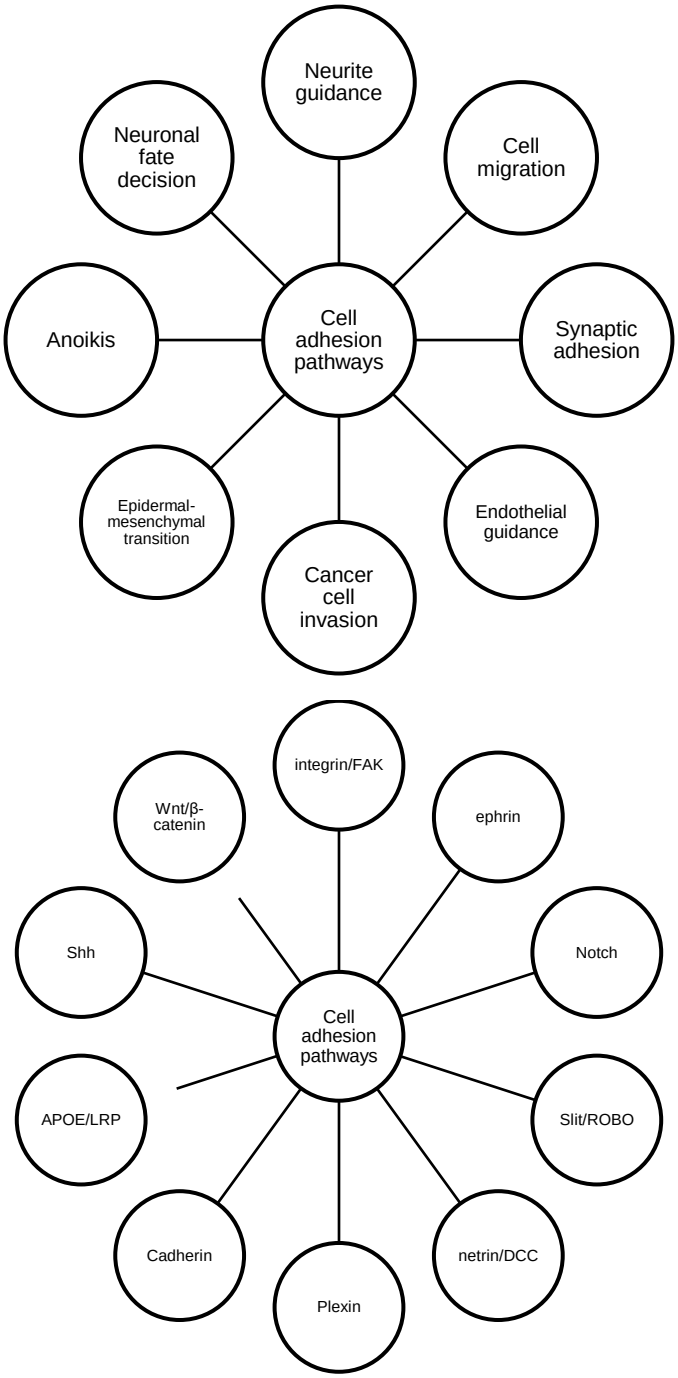
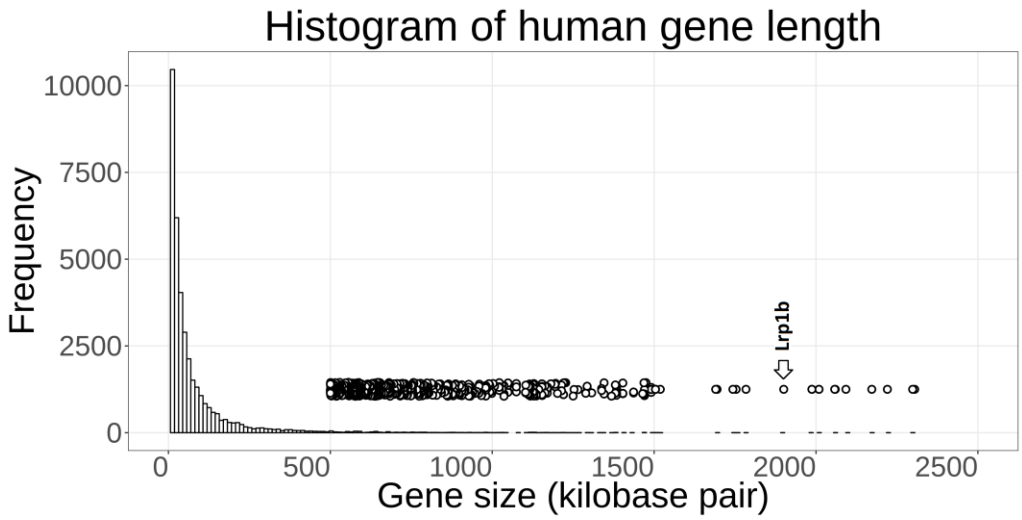


Figure 1. Top: Biological adhesion pathways transfer microenvironmental signals across the cell membrane, and affect cell polarity, movement and survival. Bottom: Various pathways of extracellular adhesion coordinate rearrangement of the actin cytoskeleton and thereby control reshaping of membrane protrusions for movement. FAK: focal adhesion kinase; LRP: lipoprotein receptor; Shh: Sonic hedgehog.

3 Aging and the Alzheimer's disease

Human aging is the strongest risk factor for dementia, but etiology of its correlation with AD remains elusive. One possibility is that AD may represent a continuation of global aging process, and cellular disruptions which happen in "normal" aging may give rise to dementia when accelerated[7]. An elegant work has recently revealed that frontal cortex cells of healthy humans accumulate ~37 new point mutations each year[206]. In line with the DNA damage hypothesis of aging, loss of genomic integrity has been previously implicated in AD predisposition[12,207,208].

From a statistical point of view, even if a purely-random and stochastic process causes 37 annual mutations in aging neurons, larger genes are expected to be disproportionately affected in late life. Considering the low rate of somatic mutations estimated in aging frontal cortex cells (5.7×10^{-9} mutations/bp.year[206]), only near one percent of the copies of a median-sized human gene will be affected by at least one somatic mutation in a 65-year individual. However, in sharp contrast, the largest known human gene, CNTNAP2, which is more than 80x larger than a median-sized protein-coding locus and codes for a synaptic adhesion molecule, is expected to be highly vulnerable to somatic mutations, and only 42 percent of its copies are estimated to remain intact at the same age (Fig. 3). This high variability is due to the characteristic distribution of human gene size parameter, which spans three-orders of magnitude with a long tail encompassing a subset of extremely large genes (Fig. 2). Intriguingly, many of the largest known human genes map to chromosomal fragile sites[209].



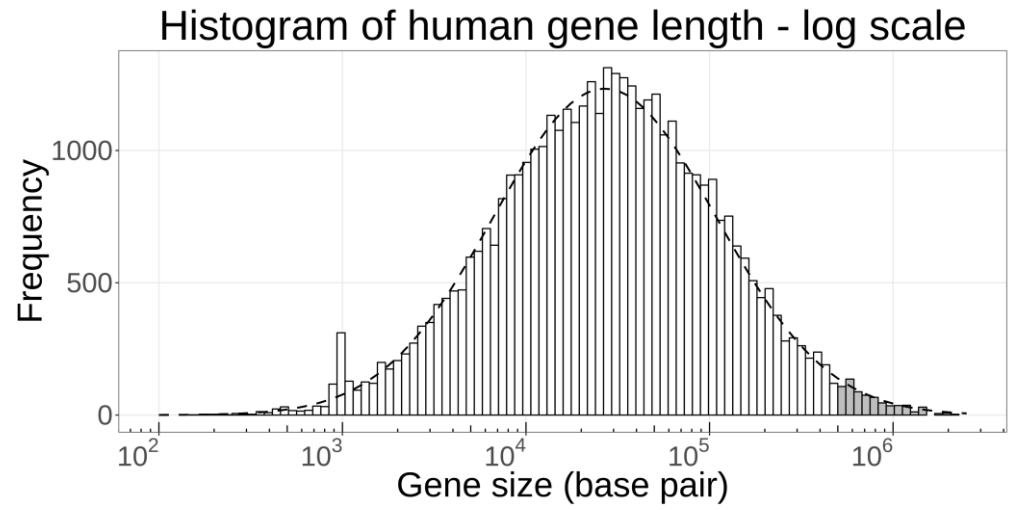


Figure 2. **Top:** Human gene length distribution has a long tail that extends towards a group of extremely-large genes in the megabase pair range. The arrow points to the giant APOE receptor, Lrp1b. **Bottom:** Human gene size parameter closely follows a log-normal distribution with parameters μ=ln(26.9kbp) and σ=1.4. The outlier bin near 1kbp represents the large family of olfactory receptors which have gone through extreme evolutionary expansion. Scattered circles (**top**) and grey bars (**bottom**) show the subset of large genes used in functional enrichment analyses (>500kbp).

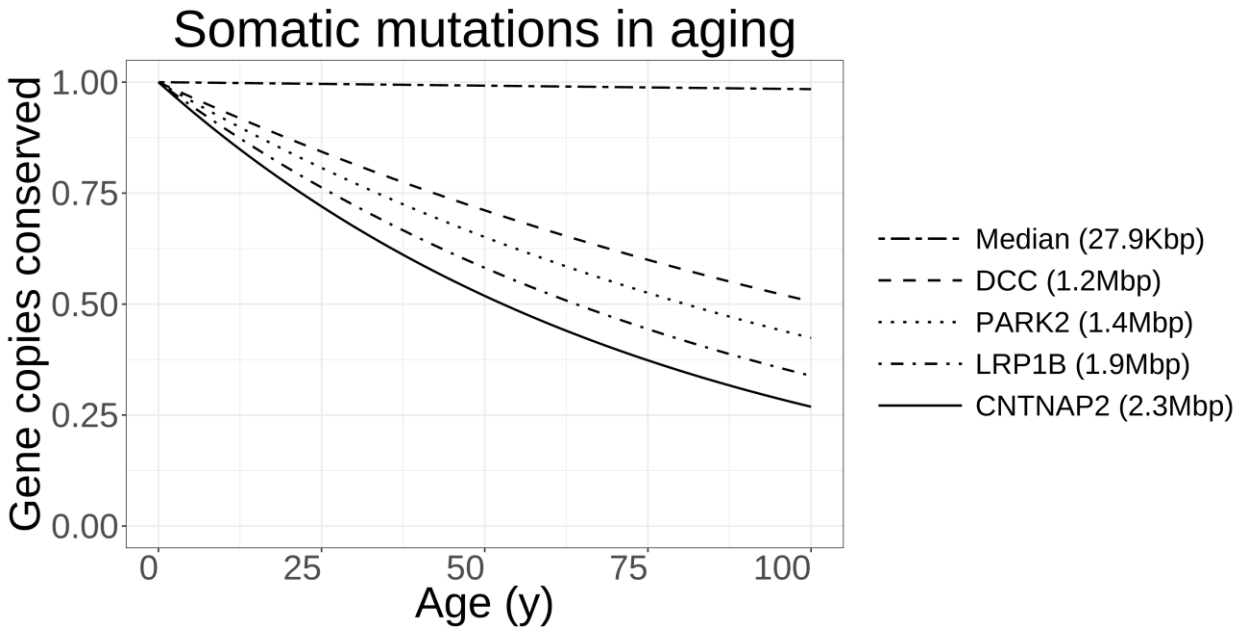


Figure 3. A binomial model in which somatic mutations take place at a fixed rate across the whole span of human genome has wildly different impacts on genes of various sizes. According to this model, a median-sized human gene mostly survives the mutational burden of aging, with only ~1 percent of its copies affected by any somatic mutation in late life. However, larger genes possess significantly shorter half-lives due to somatic mutations. Many of these large genes regulate synaptic function and integrity and are also known to act as tumor suppressors.

These clues compelled us to objectively investigate whether the largest human genes non-randomly take part in certain cellular mechanisms. We size-sorted all of the protein-coding human genes (n=18,181 genes with UniProt accession). Thereafter, a minimum gene length threshold of >500kb was considered, a cut-off filter that resulted in inclusion of 234 genes representing the top 1.3 percent of largest protein-coding loci. Functional annotation and tissue expression profile of this gene set of interest were investigated using Database for Annotation, Visualization and Integrated Discovery (DAVID) and standard statistical tests of gene set enrichment[210,211].

The most overrepresented organ for expression of this gene set was brain ($p=2.1\times10^{-17}$), followed by amygdala ($p=1.9\times10^{-4}$) and hippocampus ($p=8.5\times10^{-4}$). Showing strong enrichment statistics, *cell adhesion* (GO:0007155) was the most representative biological process related to this gene set ($p=8.0\times10^{-13}$, Table 1) and the most overrepresented cellular component was *synapse* (GO:0045202: $p=2.4\times10^{-14}$). Other significant gene ontology terms further implicated pathways of nervous system development and function, including *neuron differentiation*, *axon morphogenesis*, *axon guidance*, *cell motion*, and *synaptic transmission* (Table 2).

The strongly nonrandom selectivity of large human genes to brain, synapse and cell adhesion is a potentially enlightening observation. We suspect that these genes may have fostered adhesion and assembly of complex synaptic circuits in cognitive evolution. As an evolutionary bottleneck,

445 extremely large genes may also be inherently costlier to be maintained in late life, and may put
446 individuals at a neurobiological disadvantage when the burden of somatic mutations passes a
447 critical threshold in aging. Remarkably, potential downside of large genes in late life aging has
448 only been weakly corrected by evolutionary forces, since average human life expectancy passed
449 the 40-year milestone only two centuries ago[212]. In this regard, AD may have unmasked a DNA
450 maintenance and repair bottleneck in the elderly brain of modern humans due to the rapid
451 expansion of life expectancy.

452 While somatic mutations provide a simple explanation for synaptic adhesion failure in aging, it is
453 worth noting that extracellular adhesion pathways also form a surveillance system for continuous
454 checking of cell anchorage in solid organs. Abnormal loss of cell adhesion robustly activates a
455 specialized apoptosis program known as anoikis[213], with a number of AD risk loci controlling
456 such anoikis pathways including the reelin pathway which regulates anoikis of mesodermal
457 cells[214] and the ephrin cascade of axon guidance which moderates anoikis in cancer[215]. Integrin is
458 also a well-characterized regulator of anoikis cell death (e.g. see[216] and reviews[217,218]). The
459 interrelationship between pathways of neurite adhesion and cell survival is at a level that they
460 occasionally rely on dual-functioning *dependence* receptors, as has been determined for netrin
461 and ephrin cascades of axon guidance[219,220].

462

463 Table 1. Enrichment of large human genes (>500kbp) in *Gene ontology: biological process*
464 annotation. p-values are corrected for multiple comparisons.

GO term: biological process	***Gene count***	***Pvalue***
GO:0007155~cell adhesion	41	8.0×10^{-13}
GO:0022610~biological adhesion	41	4.0×10^{-13}
GO:0016337~cell-cell adhesion	23	3.0×10^{-9}
GO:0019226~transmission of nerve impulse	23	1.8×10^{-7}
GO:0007268~synaptic transmission	21	2.8×10^{-7}
GO:0030182~neuron differentiation	25	3.9×10^{-7}
GO:0048666~neuron development	22	3.8×10^{-7}
GO:0030030~cell projection organization	22	1.6×10^{-6}
GO:0007156~homophilic cell adhesion	14	1.6×10^{-6}
GO:0048812~neuron projection morphogenesis	17	1.7×10^{-6}
GO:0031175~neuron projection development	18	3.4×10^{-6}
GO:0048667~cell morphogenesis involved in neuron differentiation	16	7.4×10^{-6}
GO:0048858~cell projection morphogenesis	17	9.3×10^{-6}
GO:0032990~cell part morphogenesis	17	1.6×10^{-5}
GO:0007409~axonogenesis	15	1.5×10^{-5}
GO:0000904~cell morphogenesis involved in differentiation	16	4.2×10^{-5}
GO:0007267~cell-cell signaling	24	1.8×10^{-4}
GO:0000902~cell morphogenesis	18	2.2×10^{-4}
GO:0007411~axon guidance	10	6.3×10^{-4}
GO:0050808~synapse organization	8	8.0×10^{-4}
GO:0032989~cellular component morphogenesis	18	7.6×10^{-4}
GO:0050877~neurological system process	31	0.011
GO:0006928~cell motion	17	0.021
GO:0006887~exocytosis	8	0.036

465

466

467 Table 2. Enrichment of large human genes (>500kbp) in *Gene ontology: cellular component*
468 annotation. p-values are corrected for multiple comparisons.

GO term: cellular component	***Gene count***	***Pvalue***
GO:0045202~synapse	33	2.4×10^{-14}
GO:0044456~synapse part	24	1.5×10^{-10}
GO:0030054~cell junction	31	7.8×10^{-9}
GO:0005886~plasma membrane	97	1.1×10^{-8}
GO:0045211~postsynaptic membrane	15	4.3×10^{-7}
GO:0044459~plasma membrane part	63	3.8×10^{-6}
GO:0043005~neuron projection	21	3.8×10^{-6}
GO:0014069~postsynaptic density	10	2.2×10^{-5}
GO:0042995~cell projection	29	2.3×10^{-5}
GO:0042734~presynaptic membrane	7	7.0×10^{-5}
GO:0031224~intrinsic to membrane	110	1.1×10^{-4}
GO:0016021~integral to membrane	103	1.5×10^{-3}
GO:0044463~cell projection part	13	2.7×10^{-3}
GO:0031225~anchored to membrane	12	5.8×10^{-3}
GO:0019898~extrinsic to membrane	18	0.016
GO:0030424~axon	9	0.03
GO:0030425~dendrite	9	0.03

469

470

4 Predictions

AD patients may suffer faster accumulation of DNA damage and somatic mutations in their aging neurons due to a combination of heritable factors and environmental exposures. This insult eventually disrupts cellular pathways controlled by unstable genes of synaptic maintenance and neuronal survival. Derailed signaling of the APOE-lipoprotein receptor axis may be a prototypic feature of cellular dysfunction in sporadic AD (Fig. 4).

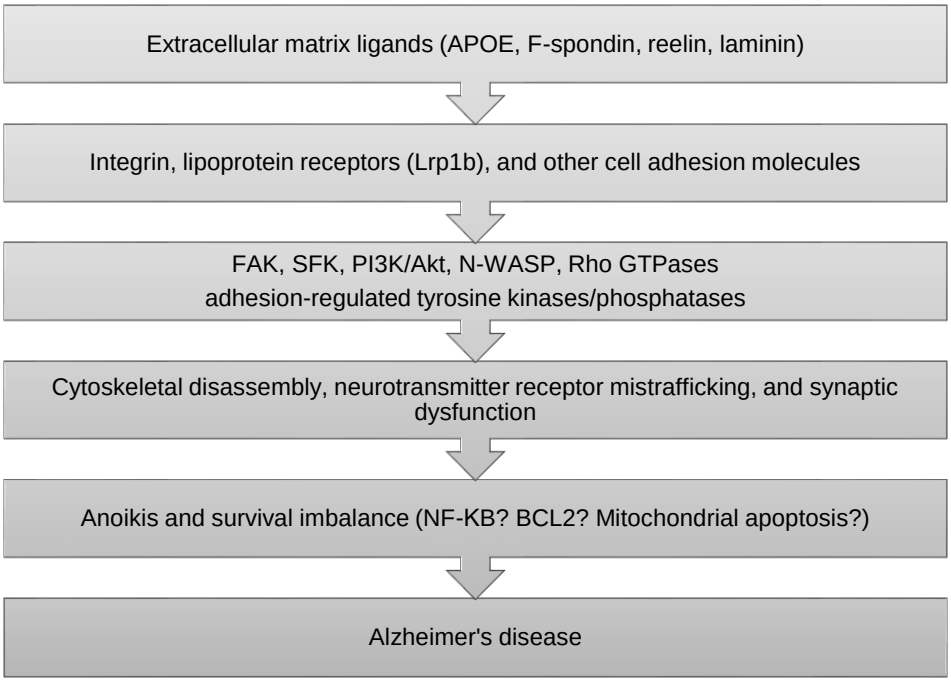


Figure 4. A simplified cascade of sporadic AD pathogenesis based on APOE signaling disruption. FAK: focal adhesion kinase; SFK: Src-family kinase.

We predict that mutational instability of the Lrp1b gene in human aging potentially explains the APOE4 risk factor of AD:

- Lrp1b is the largest member of the lipoprotein receptor family and represents the 8th largest human gene overall. Lrp1b is also one of the most frequently deleted genes in 3,131 cancer specimens and maps to a common chromosomal fragile site[223].
- Coding variants within Lrp1b are correlated with cognitive stability in aging and AD[224,225].
- Lrp1b controls focal adhesion, cytoskeletal remodeling and cell migration[226], pathways that functionally align with the genetic architecture of AD. Lrp1b has affinity to APOE particles and also interacts with APP at cell membranes, possibly acting as a co-receptor complex for cell signal transduction[227,228].

- Lrp1b interacts with two postsynaptic proteins including PSD95[134] and PICK1[229]. PSD95 is the major synaptic scaffold protein and connects glutamate receptors with the synaptic actin cytoskeleton. PICK1 regulates postsynaptic function and glutamate receptor function[230].
- Unlike other APOE receptors that are generally expressed in various organs, Lrp1b demonstrates a restrictive pattern of brain expression[227], and hippocampal CA1 neurons and amygdala show the highest Lrp1b transcription levels in the Allen human brain atlas[231] (Fig. 5).

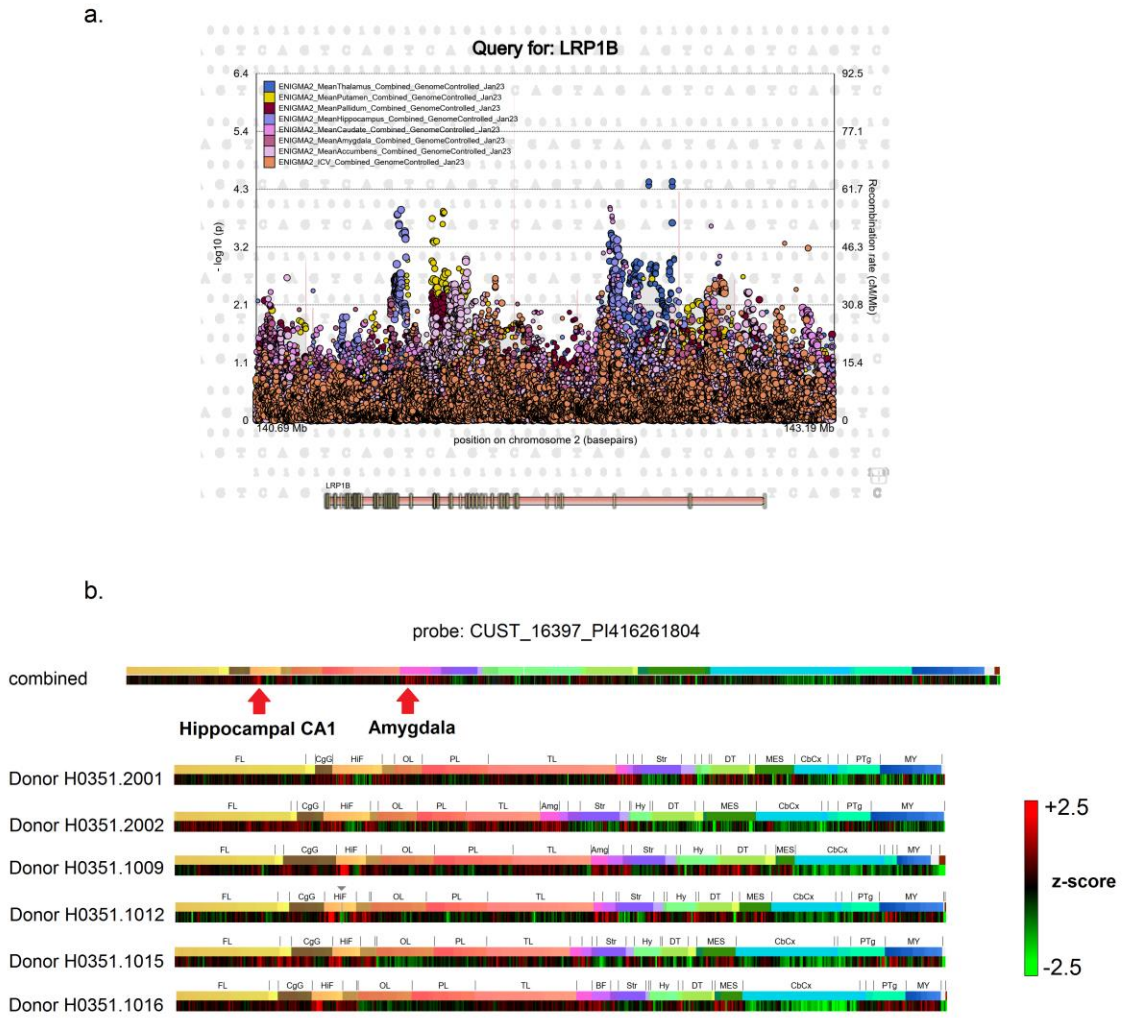


Figure 5. **Top**: Correlation of genetic polymorphisms of the Lrp1b locus with several MRI measures of brain volume in ENIGMA-2 meta-analysis[232,233] (*https://www.enigma-brain.org/enigmavis/visualizer/visualizer*). **Bottom**: Spatial expression of Lrp1b in different human brain regions in six postmortem brain samples, Allen human brain atlas (*http://human.brain-map.org*).

We predict that AD-type cognitive decline is correlated with somatic mutations in certain large synaptic genes including Lrp1b. Although previous models have already implicated oxidative stress and DNA damage in AD[12,207,208,234], high-throughput results do not support an oxidative etiology for somatic mutations in various organs, since oxidative stress typically causes G:C→T:A transversions[235,236], whereas aging cells demonstrate a clock-like signature of somatic mutations with enrichment of C:G→T:A transitions[237,238]. Intriguingly, the C:G→T:A fingerprint was recently observed as the dominant type of somatic mutations in neurons[236,239,240]. We predict that the most frequent type of genomic instability in AD may also be similar to that of "normal" aging in neurons and cancer cells. The reason for this preponderance of C:G→T:A transitions is currently unknown, and might reflect transcriptional stress, spontaneous cytosine deamination, or DNA repair failure[236,241].

It is noteworthy that Lrp1b only serves to provide one example of vulnerable genes in brain aging, and the true genetic landscape of sporadic AD and senile neurodegenerations in general is not reducible to the APOE pathway (Fig. 6). In a manner that may be similar to engagement of various tumor suppressor genes in different cancers, brain-wide expression of several unstable synaptic genes may cause heterogeneity of dementia syndromes. For instance, the genetic architecture of Parkinson's disease implicates many genes of the synaptic vesicular trafficking system including the extremely large tumor suppressor PARK2[242], and the vulnerable dopaminergic neurons selectively express two known tumor-suppressor cell adhesion genes, including DCC[243] and AJAP1[244].

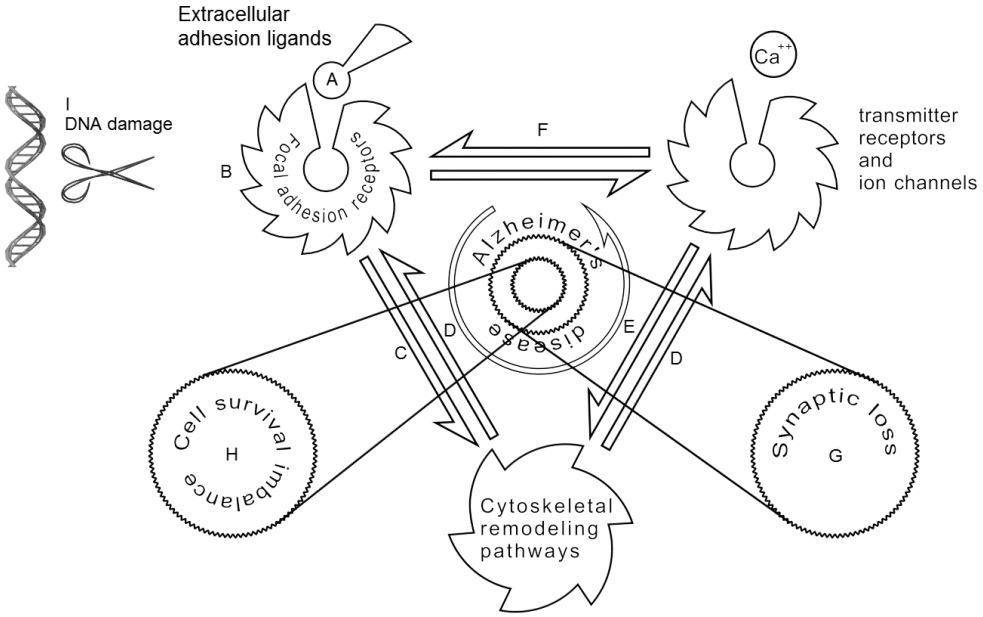


Figure 6. The proposed mechanisms of synaptic loss and neuronal death in AD. The extracellular matrix and its cell adhesion molecules (**A**) modulate neuronal adhesion receptors (**B**). Cell adhesion pathways affect remodeling of synaptic cytoskeleton as well as other modulators of

plasticity and neuronal survival, e.g. various SH3 domain containing proteins (**C**). The postsynaptic density scaffold is anchored with the synaptic actin cytoskeleton through linking proteins, e.g. PDZ domain containing proteins (**D**). Function and trafficking of the neurotransmitter receptors are controlled by cytoskeletal pathways (**E**) as well as cell adhesion molecules (**F**). Disruption of cell adhesion pathways in AD impairs synaptic stability and causes dendritic spine loss (**G**), and may eventually lead to neuronal survival imbalance by triggering anoikis-like mechanisms (**H**). Selective vulnerability of large genes in aging may be the cause of cell adhesion disruption in AD (**I**).

5 Future perspectives

Conditional knockout of Lrp1b as well as other modulators of the APOE signaling axis (F-spondin, reelin, Dab1) after completion of brain development may aid in modeling cognitive aspects of AD in laboratory animals. Intriguingly, conditional knockout of the Lrp1 gene which is closely related to Lrp1b, but lacks its brain-specific expression profile, results in neurodegenerative changes after 12 months of animal aging[245]. The single study of Lrp1b knockout mice did not follow the course of abnormal phenotypes past this age[134]. Lrp1 and Lrp1b may also be functionally redundant and partially compensate for loss of gene function in single knock-out models.

Since even the most aggressive forms of AD remain clinically-silent for decades, the short lifespan of laboratory animals may not permit effective interaction of genetic factors and environmental exposures to take place similar to human dementias. Therefore, accelerating the aging process by crossing AD animal models with DNA-repair defective strains[246] or exposure of models to genotoxic UV irradiation may prove informative, but usefulness of these gene×environment models relies on validation of DNA damage accumulation in aging human brain and its characteristics.

Our hypothesis is not based on any form of etiological relevance for Aβ species or neurofibrillary tangles in the disease cascade, and redefines these neuropathological features as bystander epiphenomena downstream to other causal factors. For AD drug design, enhancing function of the integrin-lipoprotein receptor signaling axis and their downstream second messengers including adhesion-regulated tyrosine kinases (FAK/SFK, PI3K→Akt, and Erk) or regulators of cytoskeletal actin (e.g. Rho GTPases, cofilin, and WAVE) as well as blocking anoikis pathways may prove useful. Nevertheless, even the strongest AD risk locus, APOE, fails to explain ~94 percent of the disease variance alone, and single pathway therapeutic approaches may provide limited benefit in clinical trials. As a potentially more effective method, our model warrants engineering of novel gene delivery vehicles for restoring neuronal expression of unstable genes before somatic mutations reach a level that results in clinical presentation of dementia.

In conclusion, our proposal, the *large gene instability hypothesis,* implicates mutational vulnerability of the complex genes of synaptic adhesion and homeostasis as the primary etiology

of AD, and suggests a shift of paradigm from the protein aggregation process to DNA damage mechanisms for disease prevention and drug design.

Acknowledgements

S.S. is supported by NIH Big Data to Knowledge (BD2K) grant U54 EB20403.

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