A genetic cause of Alzheimer disease: mechanistic insights from Down syndrome

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Abstract

Down syndrome, which arises in individuals carrying an extra copy of chromosome 21, is associated with a greatly increased risk of early-onset Alzheimer disease. It is thought that this risk...
is conferred by the presence of three copies of the gene encoding amyloid precursor protein (APP) — an Alzheimer disease risk factor — although the possession of extra copies of other chromosome 21 genes may also play a part. Further study of the mechanisms underlying the development of Alzheimer disease in people with Down syndrome could provide insights into the mechanisms that cause dementia in the general population.

Down syndrome (DS) is a complex, highly variable disorder that arises from trisomy of chromosome 21. It was one of the first chromosomal disorders to be identified\(^1\) and occurs with an incidence of approximately 1 in 800 births\(^2\). Its prevalence within a given population is influenced by infant mortality rates, access to health care, termination rates, average maternal age\(^3\) and life expectancy. Indeed, despite the increased availability of prenatal diagnosis and access to the option of termination, the global prevalence of DS is rising because of improvements in life expectancy: the number of adults with DS aged over 40 years has doubled in northern Europe since 1990 and, in the United Kingdom, one-third of the estimated 40,000 people with DS are thought to be over 40 years of age\(^4\).

DS is the most common form of intellectual disability. In addition to the features that are found in everyone with the disorder, such as the characteristic facial dysmorphology, there are many DS-associated phenotypes that have variable penetrance and severity. For example, approximately 40% of individuals with DS have heart malformations (usually atrioventricular septal defects)\(^5\). A key feature of DS is a striking propensity to develop early-onset Alzheimer disease (EOAD). Complete trisomy of chromosome 21 universally causes the development of amyloid plaques and neurofibrillary tangles (NFTs), which are typical characteristics of AD brain pathology, by the age of 40, and approximately two-thirds of individuals with DS develop dementia by the age of 60 (REFS 6,7). However, rates of dementia do not reach 100%, even in older individuals, suggesting that some individuals with DS are protected from the onset of AD (FIG. 1).

All of the features of DS arise because of aberrant dosages of coding and/or non-coding sequences present on chromosome 21. Among these sequences, the gene encoding amyloid precursor protein (APP) is thought to have a key role in the pathology of AD. The additional copy of APP may drive the development of AD in individuals with DS (AD-DS) by increasing the levels of amyloid-β (Aβ), a cleavage product of APP that misfolds and accumulates in the brain in people with AD. Consistent with this hypothesis, individuals with small internal chromosome 21 duplications that result in three copies of APP — a rare familial trait known as duplication of APP (Dup-APP) — also develop EOAD\(^8\)–\(^15\). Conversely, partial trisomy of chromosome 21 that does not result in the presence of an extra APP does not lead to AD\(^16\),\(^17\). Several additional genes on chromosome 21 are proposed to modulate the course of AD-DS, but further work is required to determine their role and relative importance.

The aim of this Opinion article is to present an overview of clinical and pathological features of AD-DS and, by comparing these to other forms of AD (particularly AD induced by Dup-APP), to highlight shared genetic, pathogenic and protective mechanisms and to discuss key future research areas. Similarities in the aetiologies of AD-DS and other forms of AD may highlight common disease mechanisms, whereas differences between these forms of AD...
may help to identify novel genes and pathways that are important in particular aspects of AD. Recent advances in genetic, cellular and neuroimaging technologies have provided the means to comprehensively explore the link between AD and DS, and recent improvements in the life expectancy of people who have DS mean that more individuals than ever before are developing AD-DS. The growing interest in AD-DS is long overdue, given the high AD burden in the DS population, and it is likely that research into AD-DS may also lead to a better understanding of AD in the general population.

**Prevalence of AD-DS**

A loss of cognitive function in middle-aged adults with DS was described soon after the identification of the syndrome\(^\text{18}\), and it was later shown that this loss resulted from the onset of AD dementia. As indicated above, AD is now common in adults with DS who are over the age of 40 years and, like other genetic forms of EOAD, develops two to three decades earlier in individuals with DS than in the general population. Data describing the prevalence of AD-DS vary between studies because of diagnostic issues, such as the presence of variable premorbid deficits, and survey methodology\(^\text{19}\). However, the prevalence of AD in people who have DS is <5% under the age of 40 (REF. 20) and then roughly doubles with each 5-year interval up to the age of 60. Hence, approximately 5–15% of individuals with DS aged 40–49 years and >30% of those aged 50–59 years experience significant cognitive decline, indicating dementia (FIG. 1). Thus, as with AD in the general population, age is a strong independent risk factor for AD-DS\(^\text{21}\). By the age of 65, 68–80% of individuals with DS have been shown to have developed dementia\(^\text{6,7}\) (FIG. 1; Supplementary information S1 (table)), and some studies of institutionalized people with DS suggest that rates are even higher\(^\text{6,20,22}\). However, not all older individuals with DS develop dementia, with some reaching their 70s without significant symptoms of AD despite having full trisomy of chromosome 21 (REF. 23). After the age of 60, prevalence rates decrease, probably owing to the high mortality rate that is associated with dementia\(^\text{21}\).

The average age at which menopause begins in women with DS correlates with the age of onset of dementia\(^\text{24–26}\); however, unlike the incidence of AD in euploid individuals, gender does not affect the incidence of AD-DS\(^\text{20,21}\). The reasons for this difference between the two populations are unknown, although it is possible that trisomy may cause changes in hormonal or cardiovascular biology that alter AD risk. The influence of gender on dementia development is complex in both the DS and euploid populations, and warrants more-extensive, longitudinal, population-based study.

Although increased levels of triglycerides and total body fat and low rates of exercise are reported in adults with DS\(^\text{27}\), and higher cholesterol levels have been associated with the risk of developing dementia in this group\(^\text{28}\), individuals with DS have lower rates of other cardiovascular risk factors — including hypertension, atherosclerosis and smoking\(^\text{29,30}\) — that are thought to contribute to the development of dementia in the general population\(^\text{31}\). Further studies are required to understand how trisomy alters the biology of the cardiovascular system and what impact this has on neurodegeneration in people who have DS.
The brain reserve hypothesis is based on the observation that, in the general population, individuals with higher levels of education and/or more-active social and intellectual lifestyles have a lower risk of developing dementia. The hypothesis predicts that individuals with more-severe premorbid cognitive impairment will have an increased risk of developing dementia. However, no convincing relationship between severity of intellectual disability (or intelligence quotient (IQ) score) and risk of AD has been found in people with DS, possibly because of diagnostic difficulties in those with severe impairments. Survival time for AD-DS does not differ much from that for late-onset AD (LOAD), with estimates varying between 3.5 years (s.d. 2.2) and 6.24 years (s.d. 4.1). However, individuals with severe intellectual disability and dementia were found to have a longer survival time after diagnosis than those with milder intellectual disability, further suggesting that reduced brain reserve does not accelerate disease progression in AD-DS.

Thus, people who have DS are at a greatly increased risk of developing dementia, with approximately 70% of individuals developing the condition by the age of 65. However, unlike the situation for LOAD, gender and cognitive reserve do not seem to influence AD-DS onset.

**Clinical features of AD-DS**

The early symptoms of AD-DS include features that are typical of other forms of AD, such as a decline in memory and language skills that may be present several years before dementia is diagnosed. However, changes in personality and behaviour are more common in the early stages of AD-DS than they are in other forms of AD: individuals typically display either apathy, lack of motivation and stubbornness, or increasing behavioural excesses and impulsivity. These ‘non-cognitive’ changes (also referred to as behavioural and psychological symptoms of dementia (BPSDs)) are associated with deficits in executive functioning and with the frontal atrophy that is visible on MRI scans, which may indicate frontal lobe dysfunction. These changes may be related to pre-existing deficits in the integrity of the frontal tracts that have been observed in individuals with DS and that may be worsened by Aβ deposition in the frontal lobes. Although BPSDs are very prominent in early AD-DS, this presentation is not unique to these individuals — it also occurs, albeit at lower rates, during the early stages of LOAD and EOAD, particularly in cases arising from mutations in the AD risk gene presenilin 1 (PSEN1; which maps to chromosome 14). Further studies are required to determine the earliest changes associated with the development of dementia in people who have DS, and to delineate other clinical differences between AD-DS, LOAD and familial forms of EOAD, such as the frequencies of co-morbidities that may affect the onset and progression of dementia (for example, cardiovascular disease and systemic infections).

Another feature of AD-DS is the more-frequent and earlier appearance of neurological symptoms such as gait disturbance and seizures when compared with LOAD. Although heterogeneous, seizures associated with AD-DS often initially present with myoclonic jerks before progressing to tonic–clonic seizures and later to non-epileptic myoclonus with cerebellar signs; electroencephalograms show diffuse slowing and spike-and-wave patterns. In individuals with LOAD, both complex partial and tonic–clonic seizures...
have been reported to be the predominant type\textsuperscript{51,52}. Although seizures are reported to occur in 0.5–64\% of people with LOAD\textsuperscript{51}, more-recent population studies have suggested that seizure incidence in LOAD is relatively low, occurring in <5\% of cases of the disease\textsuperscript{53}. By contrast, most people with AD-DS eventually develop seizures, and a sudden onset of seizures in older adults with DS is highly suggestive of AD. Co-morbid seizures are associated with a more-aggressive course of AD-DS\textsuperscript{54} and a greater dementia-associated mortality rate\textsuperscript{6}. The mechanism underlying this striking clinical feature of AD-DS is not understood, and the study of this may provide significant insights into neurodegeneration, in particular how changes in neuronal structure and organization affect disease progression.

Similarly to other forms of AD, the decline through middle-stage AD-DS dementia progressively affects more cognitive domains and results in symptoms such as dyspraxia, increasing incontinence and pathological grasping and sucking reflexes\textsuperscript{55,56}, as well as symptoms of parkinsonism\textsuperscript{22}. In summary, BPSDs may be an important early feature of AD-DS, and seizures are commonly associated with AD-DS. However, further comparative and mechanistic studies are required to unravel the importance of these clinical observations.

### Neuropathological changes in AD-DS

The similarity between the neuropathological changes that occur in AD-DS and those that characterize AD in other individuals was first noted in 1929 (REF. 57) and was important for the widespread recognition of dementia in people who have DS. This discovery also had a key role in the identification of $\text{A}\beta$ as the major constituent of amyloid plaques\textsuperscript{58}, the identification of the first AD gene, APP\textsuperscript{59}, and the subsequent development of the amyloid cascade hypothesis\textsuperscript{60}.

The overall distribution and biochemical composition of plaques (largely composed of $\text{A}\beta$) and NFTs (largely composed of tau protein (encoded by microtubule-associated protein tau ($\text{MAPT}$))) in people who have DS, EOAD and LOAD are similar\textsuperscript{58,61–63}. However, a greater deposition of plaques and tangles occurs in the hippocampus in AD-DS than in EOAD\textsuperscript{64} and, consistent with this, histological studies suggest that the earliest $\text{A}\beta$ deposition in AD-DS occurs in the hippocampus\textsuperscript{65}, whereas in LOAD the earliest deposition occurs in the basal cortex\textsuperscript{66}. Furthermore, a lower density of $\text{A}\beta$ plaques has been reported in the cortex in AD-DS than in LOAD\textsuperscript{64,66,68}. These differences may relate to amyloid plaques in AD-DS having a more amorphous morphology and a larger average size than those present in LOAD\textsuperscript{69,70}, resulting in a lower density caused by the presence of fewer but larger plaques. In addition, the aggregation kinetics of $\text{A}\beta$ may differ in people with DS because of a higher concentration of the peptide resulting from their additional copy of $\text{APP}$. Alternatively, differences in plaque load may result from the neurodevelopmental differences that occur in people who have DS, resulting in changes in synaptic activity, which is known to regulate $\text{A}\beta$ production\textsuperscript{71}.

In AD-DS, intracellular accumulation of $\text{A}\beta$ precedes extracellular plaque accumulation\textsuperscript{72–75} but becomes less prominent in older individuals with extensive pathology, as also observed in LOAD\textsuperscript{76}. Additionally, in AD-DS, diffuse plaques composed of non-fibrillary deposits of $\text{A}\beta$ develop before those with dense cores that are composed of amyloid (Supplementary
information S2(table))34,64,65,73,74,77–88. Diffuse plaques are typically not associated with other forms of neuropathology, such as activated glial cells or synaptic loss, whereas dense-core plaques are often associated with dystrophic neurites and activated astroglia and microgliain89. Also, Aβ42 — a form of Aβ that has a high tendency to aggregate — accumulates before deposition of Aβ40 in AD-DS73,74,80, which is consistent with the higher abundance of Aβ42 reported in plaques in other forms of AD89. Cerebral amyloid angiopathy (CAA) — the deposition of Aβ within cerebral blood vessels — is also observed in older individuals with DS74,80,87,90. However, unlike in LOAD, infarcts64 and vascular dementia seem to be rare in AD-DS91, although cases of CAA-associated cerebral haemorrhage have been described92–95.

In contrast to the findings of the histological studies described above, in vivo amyloid imaging by positron emission tomography (PET) indicates that the earliest site of Aβ accumulation in AD-DS, as in EOAD, could be the striatum96 and that enhanced deposition may occur in the frontal and parietal cortex97. This discrepancy may be because amyloid imaging recognizes only a subset of Aβ aggregates, thus not all deposition may be detected98. Nonetheless, most individuals with DS have amyloid-positive PET scans by the age of 50 (REFS 45,96,99,100). Amyloid load, as measured by PET, does not correlate well with cognitive function in adults who have DS in cross-sectional studies45,99, highlighting the importance of factors other than amyloid in the development of dementia. However, longitudinal imaging studies in this population have yet to be undertaken and may be highly informative45,99.

No NFTs have been reported in AD-DS in the absence of dense-core plaque pathology, which is consistent with the predictions of the amyloid cascade hypothesis. The density of NFTs triples between the fourth and fifth decade of life in AD-DS77, mirroring the onset of dementia, and NFT formation rather than amyloid deposition correlates best with cognitive decline34, which is consistent with similar findings in LOAD. Thus, changes in tau may result in neuronal dysfunction in both AD-DS and LOAD. Interestingly, smaller relative changes in nucleolar volume and a trend of reduced cell loss have been reported in the cortex and locus coeruleus in AD-DS compared with LOAD, despite comparable NFT loads, although similar cell loss was observed in other brain areas68. This may reflect a differential response of the trisomic CNS to accumulation of aggregated tau — suggesting, intriguingly, that chromosome 21 could encode a gene (or genes) that is neuroprotective when triplicated. Further study is required to determine whether trisomy 21 may provide protection from neurodegeneration.

As with people in the euploid population, people who have DS may have extensive amyloid deposition but no clinical signs of dementia (FIG. 1). Understanding how pathological changes due to AD relate to cognitive dysfunction is therefore a key research challenge. Identifying the processes that cause an amyloid-laden brain to convert from cognitively intact to impaired is crucial for understanding and successfully treating AD. As people who have DS develop amyloid deposition and NFTs by the age of 40, study of this group of individuals is likely to provide an important insight into the factors that cause dementia. Indeed, observations of AD-DS neuropathology already underpin our mechanistic

*Nat Rev Neurosci.* Author manuscript; available in PMC 2015 December 15.
understanding of AD, providing a detailed sequence of pathological changes and how these may relate to changes in cognition.

Pathological features other than plaques and NFTs also develop in both AD-DS and LOAD. Neuronal accumulation of ubiquitylated and aggregated transactive response DNA-binding protein 43 (TDP43; also known as TARDBP) in the cytoplasm and neurites is similar in AD-DS (7–14% of cases) and familial AD (10–14% of cases), whereas TDP43 neuropathology occurs more frequently in LOAD (29–79% of cases), perhaps because of the later disease onset. Lewy bodies, particularly in the amygdala, occur at a similar frequency in AD-DS and LOAD, but dementia with Lewy bodies (DLB), which is characterized by cognitive decline with hallucinations and parkinsonism features, is rare in DS. Granulovacular degeneration, the formation of electron-dense granules in double-membrane-bound cytoplasmic vacuoles, associated with plaque and NFT pathology occurs at a similar frequency in AD-DS and AD. How this pathology relates to the very early endosomal abnormalities that are reported to occur before birth in individuals with DS is unclear and warrants further investigation. Recent AD-related genome-wide association studies have highlighted the importance of the endosomal system in LOAD, indicating that this system may be of particular significance to disease.

**AD-DS versus Dup-APP-associated AD**

Dup-APP is a rare cause of familial EOAD, and comparison with AD-DS yields pathogenetic insights, as an additional copy of APP is present in both diseases. They therefore differ from other forms of familial AD that are the result of mutations in APP, PSEN1 or PSEN2 that modulate the processing of APP and the generation of Aβ. In Dup-APP, regions of chromosome 21 triplication vary in size (FIG. 2); the smallest known duplication contains only an additional copy of APP and no other coding genes. By contrast, in AD-DS, triplication of any chromosome 21 gene in addition to APP may modulate the development of dementia. Studying these genes may therefore provide novel insights into AD mechanisms.

The age of onset of dementia in individuals with Dup-APP ranges from 39 to 64 years (mean age ~52 years), and dementia shows virtually complete penetrance by the age of 65 years. By contrast, there is a broad variation in age of onset in AD-DS, and many individuals present with significant cognitive decline only after the age of 55 years, or even escape it altogether. This is remarkable given the usual co-morbid health issues and relative lack of brain reserve in individuals with DS. Thus, a possible protective mechanism (or mechanisms) from triplication of an unknown gene (or genes) on chromosome 21 may be important for resistance to dementia in people with DS. Moreover, intracerebral haemorrhage (ICH) is common in individuals with Dup-APP (occurring in 20–50% of cases), whereas individuals with DS are generally protected from this pathology, with only occasional reports. Thus, triplication of a chromosome 21 gene (or genes) may protect against some AD co-morbidity, and further comparative study of AD-DS and Dup-APP is required to understand the mechanisms underpinning this observation.
The few histopathological Dup-APP studies that have been carried out report diffuse atrophy with associated neuronal loss, deposition of plaques, CAA and accumulation of intraneuronal Aβ40 and NFTs1,109, and this pathology seems to be similar to AD-DS pathology (Supplementary information S3 (table)). However, further studies are needed75,109. Clinical DLB and cortical Lewy bodies have been observed in a few cases1,11,13,109, but currently there are insufficient data on these phenotypes to compare Dup-APP with AD-DS or LOAD. As in AD-DS, there is a greatly increased risk of dementia-associated seizures in Dup-APP10–13,47, in contrast to LOAD, in which seizures are relatively rare. This suggests that duplication of APP, and possibly of other genes located nearby, could be epileptogenic; however, as late-onset seizures often follow onset of dementia, they may also be related to synaptic deterioration that results in abnormal synchronization of neuronal networks and hyperexcitability110.

**Genes and mechanisms in AD-DS**

The presence of three copies of a dosage-sensitive gene (or genes) on chromosome 21 results in greatly enhanced risk of AD. Chromosome 21 carries 233 coding genes, 299 long non-coding genes (Ensembl release 78) and 29 microRNAs (miRBase release 21)111; thus, one or more of these must have a key role in AD. The phenotype resulting from a dosage-sensitive gene depends on the number of copies of the gene in the genome. However, not all genes are dosage sensitive, as homeostasis often prevents a gene from being overexpressed and the regulation of expression is often dependent on environmental context112. Furthermore, trisomy 21 causes widespread transcriptional dysregulation112,113, which may be the result of aneuploidy rather than of triplication of a specific gene. The importance of this in AD-DS remains unclear. Finally, acceleration of the epigenetic changes associated with ageing occurs in the DS brain114 — whether this alters gene expression or modulates the development of AD is an important area for future study.

The development of neuropathology and dementia varies significantly between individuals with DS, and understanding the factors (genetic or environmental) that cause this variation is likely to provide key insights into disease mechanisms. Below, we describe the genes that are currently implicated in the development of AD-DS and highlight the importance of further study of the genetics of AD-DS to understand how variation in the whole genome influences the development of disease.

**Triplication of APP**

The key dosage-sensitive gene for AD-DS is likely to be APP, as an additional normal copy of this gene is sufficient to cause EOAD in the absence of trisomy of the rest of chromosome 21 (REFS 8–15,47,107). The additional copy of APP in DS does not typically cause substantial Aβ accumulation until the second or third decade of life, although amyloid pathology has been demonstrated in a few childhood post-mortem examinations (BOX 1; FIG. 1). This lack of early Aβ accumulation may be due to APP not becoming dosage sensitive until adulthood, as suggested by both mouse and human studies115–117. However, increased levels of soluble Aβ42 are found in ~50% of trisomy 21 fetal brains118, suggesting that APP may be dosage sensitive during fetal development of individuals with DS but that this change may not be sufficient to cause extensive Aβ deposition in the developing brain.
— perhaps because of efficient clearance. Consistent with this, overexpression of APP and/or increased levels of Aβ have been reported in trisomy 21 human cell models, including in induced pluripotent stem cells (iPSCs) derived from infants or young adults with DS. Although triplication of APP does not necessarily lead to enhanced expression of APP and subsequent increase in Aβ accumulation in all contexts, overexpression of APP is strongly linked to Aβ deposition in adult life. Thus, elucidating the factors that control the regulation of APP expression will considerably aid our understanding of AD.

**Interaction of other chromosome 21 genes with APP**

Several proteins encoded by other chromosome 21 genes have been suggested to modulate APP processing and Aβ generation (BOX 2; FIG. 3). For example, the transcription factor ETS2 is thought to transactivate the APP promoter, leading to overexpression. The chromosome 21-encoded proteins small ubiquitin-related modifier 3 (SUMO3) and dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) modify APP post-translationally, which may alter Aβ generation. Additionally, the chromosome 21 microRNA miR-155 has been suggested to modulate γ-secretase activity and hence the processing of APP, through its effect on the expression of sorting nexin 27. Moreover, the β-secretase responsible for processing APP, β-site APP-cleaving enzyme 1 (BACE1), has a homologue, BACE2, encoded on chromosome 21, which may influence the onset of dementia in people with DS. BACE2 does not have β-secretase activity, and in fact cleaves APP on the carboxy-terminal side of the β-secretase cut site within the Aβ region, preventing generation of the peptide. Thus, enhancing BACE2 expression may be protective against accumulation of Aβ. However, BACE2 overexpression does not alter Aβ accumulation in a mouse model, and the protein does not seem to have enhanced expression in the adult DS brain. Whether triplication of any chromosome 21 gene alters APP biology sufficiently to modulate the development of AD remains to be determined.

**Genes involved in LOAD**

Polymorphisms in genes with important functions in LOAD have similar roles in the development of AD-DS; for example, the apolipoprotein E (APOE) ε4 allele is associated with greater Aβ deposition, as well as with earlier onset and increased risk of AD-DS, whereas the APOE ε2 allele leads to reduced Aβ deposition and a lower risk of disease. Similarly, variants of phosphatidylinositol-binding clathrin assembly protein (PICALM) and sortilin-related receptor 1 (SORL1) influence age of onset in AD-DS, as they do in LOAD, further supporting the theory that common mechanisms underlie both diseases. Whether variation in other genes with a role in LOAD is also important for AD-DS remains to be determined and is an important area for future study. Large-scale study of the genetic variants that contribute to the onset of dementia in AD-DS will provide an opportunity to gain insights into the mechanisms that underpin variation in the onset of dementia.
Disruption of secretory and endosomal systems

The earliest site of Aβ accumulation in AD-DS is within the neuron\textsuperscript{72–75}, indicating that secretory and endosomal systems are central to Aβ generation. Moreover, an extra copy of \textit{APP} is sufficient to cause endosomal enlargement and intracellular trafficking defects\textsuperscript{141,142} via an Aβ-independent mechanism\textsuperscript{143}. Enlargement of endosomes in trisomic neurons may cause axonal trafficking defects that contribute to neuronal degeneration\textsuperscript{141}.

Triplication of chromosome 21 genes other than \textit{APP} may also affect the secretory–endosome system, thereby affecting synaptic function, Aβ production and Aβ clearance. Small segmental duplications of the chromosome 21 endosome-to-Golgi-trafficking gene \textit{DOPEY2} (REF. 144) have been associated with LOAD and mild cognitive impairment\textsuperscript{14,145}, although this was not replicated in an independent study\textsuperscript{146}. A reduction in the dose of the chromosome 21 gene cystatin B (\textit{CSTB}), which encodes an endogenous inhibitor of lysosomal cathepsins, decreases the accumulation of Aβ and associated cognitive deficits\textsuperscript{147}. Overexpression of another chromosome 21 gene, synaptojanin 1 (\textit{SYNJ1}), which encodes a phosphoinositide phosphatase that regulates levels of membrane phosphatidylinositol-4,5-bisphosphate, has been associated with endosomal enlargement\textsuperscript{148}, whereas reduced expression of \textit{SYNJ1} lowers Aβ accumulation, as well as neuronal dysfunction and cognitive deficits\textsuperscript{149,150}. How endosomal enlargement caused by trisomy contributes to neuronal dysfunction and degeneration is another important area for future research.

Mitochondria and ROS

Mitochondrial dysfunction and enhanced production of reactive oxygen species (ROS) occur in people with DS and in trisomy 21 models\textsuperscript{151–154}, and may contribute to the accelerated ageing reported in people who have DS\textsuperscript{155}. Mitochondrial impairment may directly affect energy-hungry synapses, contributing to cognitive deficits\textsuperscript{156}. Moreover, increased levels of ROS make trisomic neurons more prone to undergoing apoptosis, potentially making them more likely to degenerate\textsuperscript{151}. Trisomy 21-associated increases in ROS levels may alter APP processing, promoting intracellular accumulation of Aβ\textsuperscript{119,151}. Thus, protecting the trisomic brain from ROS may be of therapeutic value, although antioxidant supplementation has failed to show efficacy in preventing dementia in this population\textsuperscript{157}. Interestingly, superoxide dismutase 1 (\textit{SOD1}), which has a key role in processing ROS, lies on chromosome 21, and upregulation of \textit{SOD1} seems to protect against APP and Aβ neurotoxicity\textsuperscript{158}, perhaps by modulating Aβ oligomerization\textsuperscript{159}. Consistent with this, higher \textit{SOD1} enzymatic activity correlates with better memory in adults with DS\textsuperscript{160}. However, increased \textit{SOD1} activity has also been suggested to cause accelerated cell senescence by increasing the levels of hydrogen peroxide, a form of ROS\textsuperscript{161}.

Neuronal development and function

Several processes are likely to contribute to the intellectual disability associated with DS. These include a reduction in the numbers of neurons and dendritic spines, dendritic arborization, an alteration in the excitatory–inhibitory balance and a global impairment in network connectivity\textsuperscript{68,162–166}. These perturbations in the structure, function and organization of the CNS may profoundly affect its degeneration in AD-DS (BOX 1).
Triplication of several chromosome 21 genes contributes to changes in neurodevelopment and/or neuronal function. For example, ubiquitin-specific peptidase 16 (USP16) or DYRK1A upregulation alters stem cell fate\textsuperscript{167–169}, which may in turn alter neuronal differentiation. Additionally, overexpression of several chromosome 21 genes (for example, the microRNA gene \textit{mir-155} and the protein-coding genes \textit{SYNJ1}, regulator of calcineurin 1 (\textit{RCAN1}), intersectin 1 (\textit{ITSN1}) and DS cell adhesion molecule (\textit{DSCAM})) has been implicated in deficits in synaptic structure and function\textsuperscript{148,170,171}. These genes may also play a part in AD-DS, perhaps via an effect on APP processing or on cognitive reserve. \textit{APP} overexpression may also affect CNS function independently of the production and accumulation of A\textsubscript{β}, because the expression level of full-length APP influences neurogenesis, neuronal migration, axonal growth and the maintenance of the excitatory–inhibitory balance\textsuperscript{172,173}. How the changes in CNS function caused by trisomy of chromosome 21 affect neurodegeneration in AD-DS is little understood and is a crucial area of future research.

**Intracellular signalling and tau**

Perturbations in intracellular signalling associated with trisomy 21 (REF. 174) may affect the response of the CNS to pathological changes. For example, overexpression of the chromosome 21 genes \textit{RCAN1} and \textit{DYRK1A} promotes aberrant phosphorylation of tau\textsuperscript{152,175–177}, \textit{DYRK1A} is dosage sensitive in the adult brain\textsuperscript{178}, and overexpression of this gene modulates tau splicing, altering the relative abundance of tau with three or four microtubule-binding domains (3-repeat (3R) and 4R tau, respectively), which may affect the formation of NFTs\textsuperscript{179,180}. Consistent with this, an increase in the ratio of 3R/4R tau has been reported to occur in AD-DS, as compared with LOAD or age-matched euploid individuals without dementia\textsuperscript{179,180}. Additionally, an increase in the total amount of tau has been reported in the cortex in AD-DS as compared with that in age-matched euploid individuals without dementia, and in DS iPSC-derived neurons\textsuperscript{122,179}; this upregulation may be the result of increased APP levels\textsuperscript{181}. \textit{DYRK1A} also downregulates the levels of neural restrictive silencing factor (NRSF; also known as REST), a neuroprotective protein\textsuperscript{168,169}, which has reduced expression in people with AD\textsuperscript{182}. Variants in \textit{DYRK1A} have been associated with risk of LOAD\textsuperscript{183}, further indicating a possible role in disease pathogenesis, although this association was not replicated in an independent study\textsuperscript{184}.

**Cholesterol metabolism**

Alterations in cholesterol metabolism may contribute to the development of dementia\textsuperscript{31}. Total cholesterol levels have been suggested to predict the onset of dementia in people with DS, particularly in those individuals who have an \textit{APOE e4} allele\textsuperscript{28}. Clinical trials are therefore underway to determine whether statins can prevent decline in older adults with DS, which may provide both clinical and mechanistic insights\textsuperscript{185}. The chromosome 21 lipid transporter ATP-binding cassette G1 (\textit{ABCG1}) has been suggested to regulate cholesterol efflux and may alter cholesterol metabolism in people with DS\textsuperscript{186}. Whether trisomy of this gene is related to the development of AD-DS remains unclear, as \textit{ABCG1} overexpression has been reported both to increase and to decrease A\textsubscript{β} generation \textit{in vitro}\textsuperscript{187,188} and does not change A\textsubscript{β} accumulation \textit{in vivo}\textsuperscript{189}, suggesting that this gene may not be associated with the development of AD-DS. Further study is required to understand the mechanisms that
underlie the link between increased cholesterol levels and the onset of dementia in individuals with DS.

**Immune system dysfunction**

Growing evidence shows that the immune system plays an important part in the development of AD. Individuals with DS are at an increased risk of immune system dysfunction: these individuals have a higher incidence of both autoimmune and infectious disease, and show upregulation of pro-inflammatory makers, including interleukin-1, in the brain. This dysregulation may contribute to AD-DS through alterations in microglial activation. Microglia in AD-DS have been reported to be associated with both mature Aβ plaques and NFTs, although the contribution of the immune response to AD-DS has yet to be fully explored. The chromosome 21 gene S100 calcium-binding protein beta (S100B) is expressed in astrocytes and is upregulated in both AD and AD-DS, and it may contribute to neurodegeneration by promoting Aβ deposition and tau phosphorylation and by creating a neurotoxic environment through the release of extracellular signals.

**Translational research**

The life expectancy of people with DS is increasing because of better health care and improved social inclusion. However, as with the euploid population, ageing brings new issues; in people with DS, a major ageing-related issue is a vastly increased risk of EOAD. People who have DS develop amyloid plaques and NFTs by the age of 40 years, and many individuals subsequently go on to develop dementia. Despite genetic and Aβ differences between the various forms of EOAD and LOAD, many similarities in disease process are observed such that AD seems to converge on common mechanisms of pathology. Thus, in the AD-DS patient population, it is feasible both to determine the factors (genetic and/or environmental) that cause conversion from pathological disease to cognitive decline and to undertake intervention trials to halt the development of dementia.

As APP gene dosage is the major determinant of AD-DS, it follows that therapies aimed at reducing Aβ (such as BACE inhibition or Aβ immunization) might have a beneficial effect in the DS population. Such approaches are being trialled for people with familial AD arising from APP or PSEN1 mutations, and similar clinical trials in AD-DS could provide valuable additional insight, given the predictable conversion to AD neuropathology and subsequent dementia in this population. Other treatment options that require further development include DYRK1A inhibitors and ROS modulators. Notably, treatment safety is of particular importance because many individuals with DS are unable to consent to their own participation in clinical trials and because they will probably need to undergo treatment for many years.

**Conclusions**

Many questions remain to be answered in AD-DS, including, most importantly, the mechanisms underlying the later onset of dementia as compared with Dup-APP, how neurodevelopmental perturbations affect neurodegeneration and the identity of any
chromosome 21 gene (or genes) that may protect against dementia. We now have a remarkable set of tools for studying AD-DS, ranging from new model systems to genomics studies. Although there are undoubtedly specific problems in both analysing and treating people who have DS for AD, such as issues of informed consent, trisomy 21 is an extremely important disorder for learning about the development of neurodegeneration and for testing potential therapeutic strategies to the benefit of everyone at risk of AD.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgements**

The authors are funded by a Wellcome Trust Strategic Award (grant number: 098330/Z/12/Z) awarded to The London Down Syndrome (LonDownS) Consortium (J.H., A.K-S., D.N., V.L.J.T. E.M.C.F. and A.S.) and the Medical Research Council (programme number U117527252; awarded to V.L.J.T.), as well as by awards from Alzheimer Research UK (awarded to F.K.W and E.M.C.F), Alzheimer Society (awarded to E.M.C.F and F.K.W.), Bailey Thomas Trust (awarded to A.S.), Epilepsy Research UK (awarded to F.K.W.), Lee Kong Chian School of Medicine, Nanyang Technological University Start-up Grant, and Singapore Ministry of Education Academic Research Fund Tier I (2014-T1-001-173; awarded to D.N).

**Glossary**

- **Dyspraxia** Disrupted fine or gross motor coordination.
- **Early-onset Alzheimer disease** (EOAD). Occurrence of Alzheimer disease before the age of 65 years.
- **Euploid** Having a normal chromosome number (46 chromosomes in 23 pairs in humans).
- **Executive functioning** Mental processing skills involving the frontal cortex; used for planning, attention focusing, working memory, mental flexibility and self-control.
- **Incidence** The rate of new occurrences of a disorder within a specified period of time.
- **Lewy bodies** Protein aggregates typically containing \( \alpha \)-synuclein.
- **Myoclonic jerks** Brief involuntary muscle twitches that are a medical sign of various neurological disorders.
- **Parkinsonism** A clinical syndrome including bradykinesia (slow movements), muscle rigidity and tremor, often due to the neurodegenerative condition Parkinson disease but also associated with other neurological conditions, toxins and medications.
- **Prevalence** The number of cases of a disorder at one time within a population.
- **Tonic–clonic seizures** A common type of epileptic seizure with a tonic phase (stiffening of muscles and loss of consciousness) followed by a clonic phase (rapid, rhythmic jerking of arms and legs).
References


Nat Rev Neurosci. Author manuscript; available in PMC 2015 December 15.


Box 1

Identifying risk and protective factors for AD in young children

It may seem counterintuitive to study infants and young children to understand a disease that presents only in adulthood. However, Alzheimer disease (AD) does not have an abrupt onset but emerges from a lengthy developmental trajectory in which precursors (for example, prodromal changes) surface well before overt dementia symptoms. Several genes involved in neurodevelopment have been suggested to have an important role in AD (including components of the WNT and reelin signalling pathway\textsuperscript{201,202}). Additionally, cultures of cells derived from infants with Down syndrome (DS) show clear overexpression of amyloid precursor protein (APP)\textsuperscript{119–122}, and amyloid-\(\beta\) (A\(\beta\)) plaques have been found in the brains of children with DS who are as young as 8 years of age\textsuperscript{65}. Thus, the syndrome offers a longitudinal perspective on the multilevel effects of A\(\beta\) and tau pathology during development.

DS is diagnosed prenatally or at birth, and all infants with DS are at a significantly increased risk of subsequently developing AD, although not all will present with dementia, even as ageing adults. It is possible that in adults with DS, patterns of individual differences between those with AD and those without AD are already rooted in their individual differences when they are just infants, at the genetic, cellular, neural, cognitive, behavioural, sleep and/or environmental levels. The challenge is to identify individual differences in childhood that pinpoint risk and protective factors for subsequent AD outcome in adulthood. We can then identify biomarkers and devise early intervention strategies, initially for individuals with DS and subsequently for members of the euploid population, revolutionizing our understanding of the pathways that lead to AD. Thus, a developmental approach is essential, especially as it has already been shown that differences that can be observed in infancy in individuals with DS (for example, in the simple planning of saccadic eye movements) have cascading effects on cognitive outcomes in childhood and adulthood (for example, on numerical processing, language and face processing)\textsuperscript{203}. Therefore, to fully comprehend AD in adults, it is crucial to study its full developmental trajectory, and understanding DS makes this possible.
Box 2

Modelling AD-DS in mice and in human iPSCs

Amyloid precursor protein (APP) overexpression in mouse models causes dysfunction of basal forebrain cholinergic neurons and synaptic and behavioural changes. However, increased expression of wild-type APP, even at levels in excess of those present in Down syndrome (DS), is insufficient to cause extensive Alzheimer disease (AD) neuropathology. Only mice expressing mutant APP and/or other AD-associated genes recapitulate aspects of AD neuropathology and/or cognitive change. Similarly, although altered expression of many chromosome 21 genes modifies mouse models of familial AD, whether a single extra copy of these genes is sufficient to affect pathology and behaviour remains unclear. However, chromosome engineering, which enables the generation of mouse models with large genomic duplications, may help to elucidate the effects of trisomy on neurodegeneration.

Reprogramming human somatic cells into induced pluripotent stem cells (iPSCs; which are in an embryonic stem cell-like state) is revolutionizing AD modelling, and advances in three-dimensional differentiation now permit the development of extensive amyloid-β (Aβ) and tau pathology in vitro. Comparisons have been made between euploid and trisomy 21 iPSCs derived from multiple sources, including different individuals (non-isogenic); isogenic lines generated in cell culture, spontaneously or by selection; lines in which one of the three copies of chromosome 21 has been silenced; monozygotic twins that were discordant for trisomy 21 (REF. 169); and non-integration-reprogrammed isogenic lines from an adult with mosaic DS (a condition in which only a percentage of an individual’s cells carry an extra copy of chromosome 21). Neurons derived from iPSCs show cellular phenotypes underpinning AD pathology, such as increased Aβ production, abnormal subcellular distribution of phosphorylated tau, mitochondrial abnormalities and accelerated cellular ageing. DS iPSC models can be used to dissect the effect of trisomy of individual chromosome 21 genes (for example, by genome editing using clustered regularly interspaced short palindromic repeat–CRISPR-associated protein 9 (CRISPR–Cas9) technology), to develop high-throughput screening assays for phenotype-correcting compounds and to investigate cellular phenotypes in iPSCs generated from individuals with DS with very early versus very late ages of onset of dementia.
The graphs show the cumulative frequency of amyloid plaque deposition (measured using histological methods and positron emission tomography with Pittsburgh compound B (PiB), a radioactive analogue of thioflavin that binds to amyloid) and neurofibrillary tangle (NFT) development (measured using histological methods), and the cumulative frequency of dementia in people with Alzheimer disease and Down syndrome (AD-DS) and in individuals with familial AD induced by duplication of amyloid precursor protein (Dup-APP). As shown, people who have DS can live for many years with substantial amyloid deposition before the development of dementia. Solid lines are based on the data described in Supplementary information S1–S3 (tables). Dashed lines indicate hypothesized development of pathology for which there are currently no data available. Further pathological and clinical studies directly comparing these two populations are required to verify the apparent differences in clinical dementia onset and to determine whether the development of pathology differs from that proposed here. Aβ, amyloid-β.
Figure 2. Regions of chromosome 21 duplicated in Dup-APP EOAD and ICH

Schematic illustrating the genetic regions affected in reported cases of early-onset Alzheimer disease (EOAD) accompanied by duplication of amyloid precursor protein (Dup-APP)\(^8\)–\(^{15,108}\). The minimal duplicated region is shown in blue: the only gene duplicated in all cases is APP. ADAMTS1, a disintegrin and metalloproteinase with thrombospondin motifs 1; ATP5J, ATP synthase-coupling factor 6; BACH1, BTB and CNC homologue 1; BTG3, BTG family member 3; C21orf91, chromosome 21 open reading frame 91; CCT8, chaperonin containing TCP1 8; CHODL, chondrolectin; CLDN17, claudin 17; CXADR, coxsackie virus and adenovirus receptor homologue; CYYR1, cysteine- and tyrosine-rich 1; GABPA, GA repeat-binding protein-alpha; GRIK1, glutamate receptor ionotropic, kainate 1; HSPA13, heat shock protein 70 kDa 13; ICH, intracerebral haemorrhage; JAM2, junction adhesion molecule 2; LIPI, lipase member I; LTN1, listerin E3 ubiquitin protein ligase 1; MAP3K7CL, MAP3K7 carboxy-terminal like; MRPL39, mitochondrial ribosomal protein L39; N6AMT1, N-6 adenine-specific DNA methyltransferase 1; NCAM2, neural cell adhesion molecule 2; NRIP1, nuclear receptor-interacting protein 1; PED, pedigree; POTED, POTE ankyrin domain family member D; RBM11, RNA-binding motif protein 11; RWDD2B, RWD domain-containing 2B; SAMSN1, SAM domain, SH3 domain and nuclear localization signals 1; TMPRSS15, transmembrane protease serine 15; USP, ubiquitin-specific peptidase.
Several genes may modulate processes that are relevant to the development of Alzheimer disease in people with Down syndrome (AD-DS); these include non-chromosome 21 genes, such as apolipoprotein E (APOE; which could alter disease by influencing cholesterol metabolism and possibly many other pathways), phosphatidylinositol-binding clathrin assembly protein (PICALM), sortilin-related receptor 1 (SORL1; which may influence disease via the endocytosis system and amyloid precursor protein (APP) processing) and microtubule-associated protein tau (MAPT). Tau aggregates to form neurofibrillary tangles.
(NFTs). Numerous chromosome 21 genes have also been suggested to influence the development of AD-DS, including genes that may influence APP processing and synaptic function via their role in the secretory–endosome system (including cystatin B (CSTB), DOPEY2, synaptojanin 1 (SYNJ1), intersectin 1 (ITSN1) and the microRNA gene mir-155), APP processing (including small ubiquitin-like modifier 3 (SUMO3), ETS2 and beta-site APP-cleaving enzyme 2 (BACE2)), cholesterol metabolism (including ATP-binding cassette G1 (ABCG1)), cellular signalling and tau phosphorylation (including dual-specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) and regulator of calcineurin 1 (RCAN1)), inflammation (including mir-155 and S100 calcium-binding protein beta (S100B)), synaptic function (including DOPEY2, SYNJ1, ITSN1, RCAN1 and mir-155), neurodevelopment (including ubiquitin-specific peptidase 16 (USP16), DYRK1A and DS cell adhesion molecule (DSCAM)) and oxidative stress (superoxide dismutase 1 (SOD1)). The relative importance of these processes to the development of dementia in AD-DS remains unclear and constitutes an area for future study. Chromosome 21 genes and gene products are shown in purple; non-chromosome 21 genes and gene products are shown in green. Aβ, amyloid-β.