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

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Abstract
Down syndrome, caused by 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 risk factor, although the possession of extra copies of other chromosome 21 genes may also play a role. Further study of the mechanisms underlying the development of Alzheimer disease in 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 identified1 and occurs with an incidence of approximately 1 in 800 births2. Its prevalence within a given population is also influenced by infant mortality rates, access to healthcare, termination rates, average maternal age3 and life expectancy.
Indeed, despite the increased availability of pre-natal 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 >40 years has doubled in Northern Europe since 1990 and, in the UK, one third of the estimated 40,000 people with DS are thought to be over 40 years of age.6

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, around 40% of individuals with DS have heart malformations (usually atroventricular septal defects).5 A key feature of DS is a striking propensity to develop early onset Alzheimer disease (AD). Complete trisomy 21 universally causes the development of both amyloid plaques and neurofibrillary tangles (NFT), typical characteristics of AD brain pathology, by age 40 and approximately two-thirds of individuals with DS develop dementia by age 60.6,7 However, rates of dementia do not reach 100% even in older individuals, suggesting that despite having an additional copy of APP throughout their lives some individuals who have DS are protected from the onset of AD (Figure 1).

All the features of DS arise due to aberrant dosages of coding and/or non-coding sequences present on chromosome 21. Among these sequences, the amyloid precursor protein gene (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 DS (AD-DS) by increasing levels of amyloid-beta (Aβ), a cleavage product of APP that misfolds and accumulates in the brain in AD. Consistent with this hypothesis, rare families with small internal chromosome 21 duplications that result in three copies of APP (known as duplication APP, or ‘Dup-APP’) also succumb to early onset AD (EOAD).8-15 Conversely, partial trisomy of chromosome 21 that does not result in 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 Perspective is to present an overview of clinical and pathological features of AD-DS and, by comparing these with other forms of AD (particularly Dup-APP), to highlight shared genetic, pathogenic and protective mechanisms and to discuss key future research areas. Similarities in the etiology of AD-DS and other forms of AD may highlight common mechanisms, and differences in these diseases may help identify novel genes and pathways important to 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 in Down syndrome**

A loss of cognitive function in middle-aged adults [Au: could you be more specific here about the ages concerned, as ‘middle-aged’ might be open to different interpretations?] with DS was described soon after the identification of the syndrome\(^{18}\) and it was later shown that this resulted from the onset of AD dementia. As indicated above, today AD is common in adults with DS over the age of 45 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\(^{19}\). However, AD prevalence in people who have DS is <5% under age 40\(^{20}\) and then roughly doubles with each 5-year interval up to the age of 60. Hence ~5-15% of those aged 40-49 and >30% of 50-59 year olds experience significant cognitive decline indicating dementia (Figure 1). Thus, as with AD in the general population, age is a strong independent risk factor for AD-DS\(^{21}\). By age 65, 68% - 80% of individuals with DS have been shown to develop dementia\(^6,7\) (Figure 1 and Supplementary Table 1), and some studies from institutionalized people with DS suggest even higher rates\(^6,20,22\). However, not all older individuals with DS develop dementia, with some reaching their 70s without significant symptoms of AD despite having full trisomy 21\(^22\). After age 60 prevalence rates decrease, likely due to the high mortality associated with dementia\(^21\).

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

Although, elevated levels of triglycerides and total body fat and low rates of exercise are reported in adults with DS\(^27\) and cholesterol levels have been associated with risk of developing dementia in this group\(^28\). Individuals with DS have lower rates of other cardiovascular risk factors, including hypertension, atherosclerosis and smoking\(^{29,30}\) that are thought to contribute to the development of
dementia in the general population\textsuperscript{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 was based upon the observation in the general population that individuals with higher levels of education and/or more active social and intellectual lifestyles had a lower risk of developing dementia\textsuperscript{32}. The hypothesis predicts that those with more severe premorbid cognitive impairment will have an increased risk of developing dementia. However, no convincing relationship between severity of intellectual disability (or IQ) and risk of AD has been found in DS\textsuperscript{33}, possibly because of diagnostic difficulties in those with severe impairments. Survival time in AD-DS does not differ much from late onset AD (LOAD), with estimates varying between 3.5 years (SD 2.2)\textsuperscript{34} and 6.24 years (SD 4.1)\textsuperscript{5}. However, those with severe intellectual disability and dementia were found to have a longer survival time after diagnoses than those with milder intellectual disability\textsuperscript{6}, further suggesting that reduced brain reserve does not accelerate disease progression in AD-DS.

People who have DS are a greatly increased risk of developing dementia, with around 70\% of individuals developing the condition by the age of 65, interestingly gender and cognitive-reserve do not appear to influence AD-DS onset, unlike in LOAD.

**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\textsuperscript{35-37}. However, changes in personality and behavior 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 behavioral excesses and impulsivity. These “non-cognitive” changes (also referred to as the behavioral and psychological symptoms of dementia, BPSD)\textsuperscript{38-42} are associated with deficits in executive functioning and frontal atrophy on MRI scans that may indicate frontal lobe dysfunction\textsuperscript{40, 43}. These changes may be related to pre-existing deficits in the integrity of the frontal tracts that have been observed in individuals with DS\textsuperscript{44} and that may be worsened by Aβ deposition in the frontal lobes\textsuperscript{45}. Although BPSD symptoms is very prominent in early AD-DS, this presentation is not unique – it also occurs albeit at lower rates during the early stages of LOAD\textsuperscript{46} and EOAD, particularly in cases arising from mutations in the AD risk gene PSEN1\textsuperscript{47} (which maps to chromosome 14). Further studies are required to determine the earliest changes
associated with 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 comorbidities (e.g. cardiovascular disease or systemic infections) that may affect the onset and progression of dementia.

Another feature of AD-DS is the more frequent and earlier appearance of neurological symptoms such as gait disturbance and seizures when compared to LOAD. Although heterogeneous, seizures associated with AD-DS often present initially with myoclonic jerks before progressing to tonic-clonic seizures and later to non-epileptic myoclonus with cerebellar signs; electro-encephalograms show diffuse slowing and spike-wave patterns. In LOAD both complex-partial and tonic-clonic seizures have been reported to be the predominant type. Although seizures are reported to occur in between 0.5%- 64 % LOAD of cases, more recent population studies have suggested seizure incidence in LOAD is relatively low, occurring in <5% of cases. In 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 and greater dementia-associated mortality. The mechanism underlying this striking clinical feature of AD-DS is not understood, and the study of this may provide significant insight 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 involves progressively more areas of cognitive function and results in symptoms such as dyspraxia, increasing incontinence, pathological grasping and sucking reflexes, and Parkinsonian symptoms. In summary, BPSD features may be an important early feature of AD-DS and seizures are commonly associated with disease but further comparative and mechanistic studies are required to unravel the importance of these clinical observations.

**AD-DS neuropathological changes**

The similarity between the neuropathological changes that occur in AD-DS and those that characterize AD was first noted in 1929, 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 Aβ as the major constituent of amyloid plaques, identification of the first AD gene, APP, and the subsequent development of the amyloid cascade hypothesis.
The overall distribution and biochemical composition of plaques (largely Aβ) and neurofibrillary tangles (NFT, largely composed of tau protein) in people who have DS, EOAD and LOAD is similar\textsuperscript{59, 62-64}. However, a greater deposition of plaques and tangles occurs in the hippocampus in AD-DS compared with EOAD \textsuperscript{65} and, consistent with this, histological studies suggest that Aβ deposition in the hippocampus occurs early in AD-DS\textsuperscript{66}, whereas in LOAD earliest deposition is within the basal cortex\textsuperscript{67}. Furthermore, a lower density of Aβ plaques has been reported in the cortex in AD-DS than LOAD \textsuperscript{68, 69}. 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{70, 71}, resulting in a lower density caused by the presence of fewer but larger plaques. In addition, the aggregation kinetics of Aβ may differ in DS because higher concentration of the peptide resulting from the additional copy of 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 Aβ production\textsuperscript{72}.

In AD-DS, intracellular accumulation of Aβ precedes extracellular plaque accumulation\textsuperscript{73-76} but becomes less prominent in older individuals with extensive pathology as also observed in LOAD\textsuperscript{77}. In AD-DS diffuse plaques formed of non-fibrillary deposits of Aβ develop prior to those with dense-cores that are composed of amyloid (\textit{Supplementary Table 2}\textsuperscript{24, 65, 66, 74, 75, 78-89}). Diffuse plaques are typically not associated with other forms of neuropathology such as activated glia cells or synaptic loss, whereas dense-cored plaques are often associated with dystrophic neurites and activated astroglia and microglia\textsuperscript{90}. Also, Aβ42 -- a form of Aβ that has a high tendency to aggregate - accumulates before deposition of Aβ40 in AD-DS \textsuperscript{74, 75, 81}, consistent with the higher abundance of Aβ42 reported in plaques in other forms of AD\textsuperscript{90}. Cerebral amyloid angiopathy (CAA) -- deposition of Aβ within cerebral blood vessels -- is also observed in older individuals with DS\textsuperscript{75, 81, 88, 91}. However in contrast to LOAD, infarcts\textsuperscript{65} and vascular dementia appear rare in AD-DS\textsuperscript{92}, although cases of CAA-associated cerebral haemorrhage have been described\textsuperscript{93-96}.

In contrast to the findings of histological studies described above, \textit{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 striatum\textsuperscript{97}, and that enhanced deposition may occur in the frontal and parietal cortex\textsuperscript{98}. This discrepancy may reflect the fact that amyloid-imaging only recognises a subset of Aβ aggregates; thus not all deposition may be detected\textsuperscript{99}. Nonetheless, most individuals with DS have amyloid positive PET scans by the age of 50\textsuperscript{65, 97, 100, 101}. Amyloid load as measured by PET does not correlate well with cognitive function in adults who have DS in cross-sectional studies\textsuperscript{45, 100}, high-
lighting 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 informative\textsuperscript{45, 100}.

No NFT 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 4\textsuperscript{th} and 5\textsuperscript{th} decade of life in AD-DS\textsuperscript{78}, mirroring the onset of dementia and NFT formation, rather than amyloid deposition\textsuperscript{34}, 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 for 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 areas\textsuperscript{69}. This may reflect a differential response of the trisomic CNS to accumulation of aggregated tau – suggesting, intriguingly, that chromosome 21 could encode gene(s) that are neuroprotective when triplicated. Further study is required to determine whether trisomy 21 may provide protection from neurodegeneration.

As in the euploid population, people who have DS may have extensive amyloid deposition, yet do not show clinical signs of dementia (Figure 1). Understanding how AD pathological changes 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 demented is crucial to 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 significant insight into the factors that cause dementia. Indeed, observations of AD-DS neuropathology already underpin our mechanistic 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 NFT also develop in both AD-DS and LOAD. Neuronal accumulation of ubiquitinated and aggregated transactive response DNA binding protein-43 (TDP43) in cytoplasm and neurites is similar in AD-DS (7-14 % cases) and familial AD (10-14%), whereas TDP43 neuropathology occurs more frequently in LOAD (29-79%), perhaps because of the later disease onset\textsuperscript{102, 103}. Lewy bodies, particularly in the amygdala, occur in AD-DS at a similar frequency to LOAD\textsuperscript{104}, but dementia with Lewy bodies (DLB), characterised by cognitive decline with hallucinations and Parkinsonism features, is rare in DS\textsuperscript{105}. Granulovacuolar degeneration, the formation of double membrane-bound electron-dense granules cytoplasmic vacuoles, associated
with plaque and NFT pathology occurs in DS-AD with a similar frequency to AD\textsuperscript{65}. How this pathology relates to the very early endosomal abnormalities reported to occur prior to birth in individuals with DS\textsuperscript{106} is unclear and warrants further investigation. Recent AD-genome wide association studies (GWAS) have highlighted the importance of the endosomal system to LOAD\textsuperscript{107} indicating that this system may be of particular importance to disease.

Comparing AD-DS and Dup-APP

Dup-APP is a rare cause of familial EOAD, and comparison to AD-DS yields pathogenetic insights, as in both diseases, an additional copy of APP is present. They therefore differ from other forms of familial AD that are the result of mutations in the APP, PSEN1 or PSEN2 genes that modulate processing of APP and generation of Aβ. In Dup-APP, regions of chromosome 21 triplication vary in size\textsuperscript{9-15, 47, 108, 109} (Figure 2) and the smallest known duplication contains only an additional copy of APP and no other coding genes\textsuperscript{8}. 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 insight into AD mechanisms.

The age of onset of dementia in Dup-APP ranges from 39 to 64 years (mean age \~52) and shows virtually complete penetrance by age 65. By contrast, AD-DS appears to have a broad variation in age of onset, and many individuals only present with significant cognitive decline after age 55, or even escape it altogether. This is remarkable given the DS co-morbid health issues and relative lack of brain reserve. Thus, a possible protective mechanism(s) from triplication of unknown gene(s) on chromosome 21 may be important for resistance to dementia in DS. Moreover, intracerebral haemorrhage is common in APP-Dup (20-50% of cases)\textsuperscript{9-14, 47, 109}, whereas individuals with DS are generally protected from this pathology with only occasional reports. Thus, triplication of a chromosome 21 gene(s) may protect against some AD-comorbidity, and further comparative study of AD-DS and APP-Dup 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, intraneuronal Aβ40 accumulation and NFT\textsuperscript{11, 110} and appear similar to AD-DS pathology (Supplementary Table 3). However, further studies are needed\textsuperscript{76, 110}. Clinical DLB and cortical Lewy bodies have been observed in a few cases\textsuperscript{11, 13, 110}, but currently there are insufficient data on these phenotypes to compare Dup-APP with AD-DS or LOAD. As in AD-DS, a greatly elevated risk of dementia associated seizures occurs in Dup-APP\textsuperscript{10-13, 47}, in contrast to LOAD in which seizures are relatively rare. This suggests that duplication of APP, and
possibly other gene(s) located nearby, could be epileptogenic; however, as late onset seizures often follow onset of dementia, they may also relate to synaptic deterioration, resulting in abnormal synchronisation of neuronal networks and hyperexcitability\textsuperscript{111}.

**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)\textsuperscript{112}; thus, one or more of these must have a key role in AD. The phenotype resulting from a dosage sensitive gene depends upon 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 over-expressed, and the regulation of expression is often dependent upon environmental context\textsuperscript{113}. Furthermore, trisomy 21 causes wide-spread transcriptional dysregulation\textsuperscript{113,114} which may be the result of aneuploidy rather than triplication of a specific gene. The importance of this to AD-DS remains unclear. Finally, acceleration of the epigenetic changes associated with aging occur in the DS brain\textsuperscript{115} -- whether this alters gene expression or modulates the development of AD is an important area for future study.

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 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\textsuperscript{8-15, 47, 108}. The additional copy of APP in DS does not typically cause substantial Aβ accumulation until the 2\textsuperscript{nd} or 3\textsuperscript{rd} decade of life, although amyloid pathology has been demonstrated in a few childhood post-mortem cases (Figure 1, Box 1). This lack of early Aβ accumulation may be because APP does not become dosage sensitive until adulthood, as suggested by both mouse and human studies\textsuperscript{116-118}. However, increased levels of soluble Aβ42 are found in ~50% of trisomy 21 fetal brains\textsuperscript{119}, suggesting that APP may be dosage sensitive during DS fetal development 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, over-expression 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\textsuperscript{120-123}. Although triplication of APP does not necessarily lead to enhanced expression of APP protein and subsequent elevation of Aβ accumulation in all contexts, overexpression of APP is strongly linked with Aβ deposition in adult life. Thus, elucidating the factors that control the regulation of APP expression will significantly aid our understanding of AD.

\textit{Interaction of other chromosome 21 genes with APP}

A number of the proteins encoded by other chromosome 21 genes have been suggested to modulate APP processing and Aβ generation (Box 2, Figure 3). For example, the transcription factor ETS2 is thought to transactivate the APP promoter, leading to over-expression\textsuperscript{124}. The chromosome 21 encoded proteins SUMO3 and DYRK1A modify APP post-translationally, which may alter Aβ generation\textsuperscript{125-127}. Additionally, the chromosome 21 microRNA, Mir155, has been suggested to modulate γ-secretase activity and hence the processing of APP, via its effect on the expression of sorting nexin 27\textsuperscript{128}. Moreover, the β-secretase responsible for processing APP, BACE1, has a homologue BACE2 encoded on chromosome 21, which may influence the onset of dementia in people with DS\textsuperscript{129}. BACE2 does not possess β-secretase activity, and in fact cleaves APP C-terminal 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\textsuperscript{130}. However, BACE2 overexpression does not alter Aβ accumulation in a mouse model\textsuperscript{131}, and the protein does not appear to have enhanced expression in the adult DS brain\textsuperscript{116, 132}. Whether triplication of any chromosome 21 gene alters APP biology sufficiently to modulate the development of AD remains to be determined.

\textit{Genes involved in LOAD}

Polymorphisms in genes with important functions in LOAD play similar roles in the development of AD-DS; for example, the APOE ε4 allele is associated with greater Aβ deposition, earlier onset and increased risk of AD-DS, whereas the APOE ε2 allele leads to reduced Aβ deposition and a lower risk of disease\textsuperscript{133-139}. Similarly, variants in PICALM and SORL1 influence age of onset in AD-DS, as they do in LOAD\textsuperscript{133, 140, 141}, 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 novel insight into the mechanisms that underpin variation in the onset of dementia.

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

Triplication of chromosome 21 genes, other than APP, may also affect the secretory-endosome system, thereby impacting synaptic function, Aβ production and Aβ clearance. Small segmental duplications of the chromosome 21 endosome to Golgi trafficking gene, DOPEY2, has been associated with LOAD and mild cognitive impairment, although this was not replicated in an independent study. A reduction in gene dose of the chromosome 21 gene, CSTB, an endogenous inhibitor of lysosomal cathepsins, decreases the accumulation of Aβ and associated cognitive deficits. Over-expression of another chromosome 21 gene, SYNJ1, a phosphoinositide phosphatase that regulates levels of membrane phosphatidylinositol-4,5-bisphosphate, has been associated with endosomal enlargement, whereas reduced expression of SYNJ1 lowers Aβ accumulation, as well as neuronal dysfunction and cognitive deficits. How endosomal enlargement, caused by trisomy, contributes to neuronal dysfunction and degeneration is another important area for future research.

Mitochondria and reactive oxygen species
Mitochondrial dysfunction and enhanced production of reactive oxygen species (ROS) occurs in people with DS and in trisomy 21 models, and may contribute to the accelerated aging reported in people who have DS. Mitochondrial impairment may directly affect energy-hungry synapses, contributing to cognitive deficits. Moreover, elevated levels of ROS make trisomic neurons more prone to undergo apoptosis, potentially making them more likely to degenerate. Trisomy 21 elevated ROS may alter APP processing, promoting intracellular accumulation of Aβ. Thus, protecting the trisomic brain from ROS may be of therapeutic value, although anti-oxidant supplementation has failed to show efficacy in preventing dementia in this population. Interestingly, superoxide dismutase 1 (SOD1), which has a key role in processing ROS, lies on chromosome 21, and up-regulation of SOD1 appears to protect against APP/Aβ neurotoxicity, perhaps by modulating Aβ oligomerisation. Consistent with this, SOD1 enzymatic activity correlates with better memory in adults with DS. However, increased SOD1 has also been suggested to cause accelerated cell senescence by elevating H₂O₂, a form of ROS.

Neuronal development and function
A number of 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 arborisation, an alteration in the excitatory-inhibitory balance and a global impairment in network connectivity\textsuperscript{69, 163-167}. These perturbations in the structure, function and organisation 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, USP16 or DYRK1A up-regulation alters stem cell fate\textsuperscript{168-170} which may in turn alter neuronal differentiation. Additionally, over-expression of several chromosome 21 genes, for example, microRNA \textit{Mir155}, and the protein coding genes \textit{SYN1}, \textit{RCAN1}, \textit{ITSN1} and \textit{DSCAM}, has been implicated in deficits in synaptic structure and function\textsuperscript{149, 171, 172}. These genes may also play a role in AD-DS, perhaps via an impact on APP processing or on cognitive reserve. \textit{APP} over-expression may also affect CNS function, independent of the production and accumulation of Aβ, because the expression level of full-length APP influences neurogenesis, neuronal migration, axonal growth, and the maintenance of the excitatory-inhibitory balance\textsuperscript{173, 174}. How the changes in CNS function caused by trisomy of chromosome 21 affect AD-DS neurodegeneration is little understood, and is a crucial area of future research.

\textit{Intracellular signalling and tau}

Perturbations in intracellular signalling associated with trisomy 21\textsuperscript{175} may affect the response of the CNS to pathological changes. For example, over-expression of the chromosome 21 genes regulator of calcineurin 1 (\textit{RCAN1}) and the kinase encoded by \textit{DYRK1A}, promotes aberrant phosphorylation of tau\textsuperscript{153, 176-178}. \textit{DYRK1A} is dosage sensitive in the adult brain\textsuperscript{179}, and overexpression of this gene modulates tau splicing, alternating the relative abundance of tau with 3 or 4 microtubule binding domains (3R/4R tau), which may affect the formation of NFTs\textsuperscript{180, 181}. 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 aged-matched non-demented euploid individuals\textsuperscript{180, 181}. Additionally, an increase in the total amount of Tau has been reported in AD-DS cortex as compared with aged-matched non-demented euploid individuals, and in DS IPSC derived neurons\textsuperscript{123, 180}, this up-regulation may be the result of increased APP\textsuperscript{182}. \textit{DYRK1A} also down-regulates the levels of neural restrictive silencing factor (NRSF/REST), a neuro-protective protein\textsuperscript{169, 170}, which has reduced expression in AD\textsuperscript{183}. Variants in \textit{DYRK1A} have been associated with risk of LOAD\textsuperscript{184}, further indicating a possible role in disease pathogenesis, although this association was not replicated in an independent study\textsuperscript{185}.

\textit{Cholesterol metabolism}
Alteration 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} \(\varepsilon4\) allele\textsuperscript{28}. Clinical trials are therefore under way to determine whether statins can prevent decline in older adults with DS, which may provide both clinical and mechanistic insight\textsuperscript{186}. The chromosome 21 lipid transporter ABCG1 has been suggested to regulate cholesterol efflux, and may alter cholesterol metabolism in DS\textsuperscript{187}. Whether trisomy of this gene is related to the development of AD-DS remains unclear, as ABCG1 over-expression has been reported to both increase and decrease A\(\beta\) generation \textit{in vitro}\textsuperscript{188, 189}, and does not change A\(\beta\) accumulation \textit{in vivo}\textsuperscript{190}, 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 elevated cholesterol and the onset of dementia in DS.

\textit{Immune system dysfunction}

Growing evidence shows that the immune system plays an important role in the development of AD\textsuperscript{107, 191}. Individuals with DS are at increased risk of immune system dysfunction, having a higher incidence of both autoimmune and infectious disease\textsuperscript{192} and an up-regulation of pro-inflammatory makers, including IL-1 in the brain\textsuperscript{193, 194}. This dysregulation may contribute to AD-DS through alterations in microglial activation\textsuperscript{191}. Microglia in AD-DS have been reported to be associated with both mature A\(\beta\) plaques\textsuperscript{195} and NFT\textsuperscript{196}, although the contribution of the immune response to AD-DS has yet to be fully explored. The chromosome 21 gene, \textit{S100B}, is expressed in astrocytes and is upregulated in both AD\textsuperscript{197} and AD-DS\textsuperscript{193}, and may contribute to neurodegeneration by promoting A\(\beta\) deposition\textsuperscript{198}, tau phosphorylation\textsuperscript{199} and creating a neuro-toxic environment via the release of extracellular signals\textsuperscript{200}.

\textit{Translational research}

The lifespan of people with DS is increasing because of better healthcare and improved social inclusion. However, as with the euploid population, ageing brings new issues and, in people with DS, this is a vastly increased risk of EOAD. People who have DS develop amyloid plaques and NFTs by the age of 40 and many individuals subsequently go on to develop dementia. Despite genetic and A\(\beta\) differences between the various forms of EOAD and LOAD, many similarities in disease process are observed such that AD appears to converge on common mechanisms of pathology. Thus, in the AD-DS patient population, it is feasible to both determine the factors (genetic 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 in DS, it follows that therapies aimed at reducing Aβ (such as BACE inhibition or Aβ immunisation) 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\(^\text{201}\), 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 treatment will likely need to be taken for many years.

**SUMMARY**

Many questions remain to be answered in AD-DS including, most importantly, the mechanisms underlying the later onset of dementia as compared with APP-dup, how neurodevelopmental perturbations impact neurodegeneration and the identity of chromosome 21 gene(s) 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. While 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.
Reference List


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Competing interests:

Dr André Strydom has participated in clinical trials sponsored by Roche of medication to ameliorate some of the co-morbidities associated with Fragile X syndrome and Down syndrome. He has acted as an adviser to the UK Down Syndrome Association (DSA) and is an advisory board member of the LuMind Foundation (USA). None of the other authors has competing interests.

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Further information

Details regarding the LonDowns Consortium can be found on this website:
http://www.ucl.ac.uk/london-down-syndrome-consortium

Box 1. Identifying risk and protective factors for AD in young children

It may seem counter-intuitive to study infants and young children to understand a disease that only presents in adulthood. However, 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, for example, components of the Wnt and Reelin signalling pathway202, 203). Additionally, cultures of cells derived from infants with DS show clear over-expression of amyloid precursor protein (APP)120-123, and Aβ plaques have been found in the brains of children with DS that are as young as 8 years of age66. Thus, the syndrome offers a longitudinal perspective on the multi-level impact of Aβ and tau pathology over development.

DS is diagnosed prenatally or at birth, and all infants with DS are at significantly increased risk of subsequently developing AD, although not all will present with dementia even as ageing adults. It is
possible that patterns of individual differences in adults with DS, with or without AD, are already rooted in individual differences in infants with DS, 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 the euploid population, revolutionising our understanding of the pathways to AD. Thus, a developmental approach is essential, especially as it has already been shown that differences that can be observed in infancy in those with DS (for example, in the simple planning of saccadic eye movements) have cascading effects on cognitive outcomes in childhood and adulthood (for example, numerical processing, language, face processing). 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 human iPS cells

Amyloid precursor protein (APP) over-expression in mouse models causes dysfunction of basal forebrain cholinergic neurons (BFCNs) and synaptic and behavioural changes. However, increased expression of wildtype APP, even at levels in excess of those present in DS, is insufficient to cause extensive 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 elucidate the effects of trisomy on neurodegeneration.

Reprogramming human somatic cells into hiPSCs (human induced pluripotential cells, an ES cell-like state) is revolutionising AD modelling, and advances in 3-D differentiation now permit development of extensive Aβ and tau pathology in vitro. Comparisons have been made between euploid and trisomy 21 hiPSCs derived from multiple sources including: from different individuals (non-isogenic); from isogenic lines generated in cell culture, spontaneously or by selection; from lines in which one of the three chromosomes 21 has been silenced; from monozygotic twins that were discordant for trisomy 21; from non-integration-reprogrammed isogenic lines from an adult with mosaic DS. Neurons derived from hiPSCs show cellular phenotypes underpinning AD pathology, for example, increased Aβ production, abnormal sub-cellular distribution of phospho-tau,
mitochondrial abnormalities and accelerated cellular aging. DS hiPSC models can be used to dissect the effect of trisomy of individual chromosome 21 genes, for example, by genome editing using CRISPR-Cas9 technology, to develop high-throughput screening assays for phenotype-correcting compounds, and to investigate cellular phenotypes in hiPSCs generated from individuals with DS with very early versus very late ages-of-onset of dementia.

**Figure 1. Development of pathology and dementia in AD-DS and Dup-APP**

The graphs show the cumulative risk of amyloid plaque deposition (measured using histological methods and positron emission tomography (PET) using Pittsburgh compound B, a radioactive analogue of Thioflavin that binds to amyloid), neurofibrillary tangles (NFT, measured using histological methods) and the cumulative frequency of dementia in people with trisomy 21 (AD-DS) compared with those in individuals with APP-duplication familial AD (AD-DupAPP). As shown, people who have DS can live for many years with substantial amyloid deposition prior to the development of dementia. Solid lines are based on data in Supplementary Tables 1-3. Dotted lines indicate hypothesised development of pathology for which there is currently no data available. Further pathological and clinical studies directly comparing these two patient populations are required to verify the apparent differences in clinical onset and to determine if the development of pathology differs from that proposed here.

**Figure 2. Regions of chromosome 21 duplicated in Dup-APP EOAD**

Schematic illustrating APP-duplication EOAD cases, showing minimal duplicated region (green). These data indicate that the only gene duplicated in all cases is APP. Data from references 8-15, 109

**Figure 3. Schematic of suggested mechanisms important in AD-DS and related genes**

A number of genes may modulate processes relevant to the development of AD-DS; these include non-chromosome 21 genes such as APOE (which could alter disease via cholesterol metabolism and possibly many other pathways), PICALM and SORL1 (which may influence disease via the endocytosis system and APP processing) and MAPT (the genes that encode tau, which aggregates to form neurofibrillary tangles (NFT)). A number of chromosome 21 genes have also been suggested to influence the development of AD-DS, including genes which may influence APP processing and
synaptic function via their role in the secretory-endosome system (CSTB, DOPEY2, SYNJ1, ITSN1, Mir155), APP processing (SUMO3, ETS2, BACE2), cholesterol metabolism (ABCG1), cellular signalling and tau phosphorylation (DYRK1A, RCAN1), inflammation (Mir155, S100B), synaptic function (DOPEY2, SYNJ1, ITSN1, RCAN1, Mir155), neurodevelopment (USP16, DYRK1A, DSCAM), and oxidative stress (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 orange, non-chromosome 21 genes and gene products are shown in red.

**Glossary Box**

**Dyspraxia.** Disrupted fine or gross motor coordination

**Early onset Alzheimer’s disease (EOAD).** Occurrence of Alzheimer’s disease before the age of 65

**Euploid.** Having a normal chromosome number (46 chromosomes in 23 pairs in humans)

**Executive functioning.** Executive function skills are mental processes involving frontal cortex, for planning, focusing attention, 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.** A medical sign; brief involuntary muscle twitches

**Parkinsonism/ Parkinsonian symptoms.** Clinical syndrome including bradykinesia (slow movements) muscle rigidity and tremor, often due to the neurodegenerative condition Parkinson’s disease, but also associated with other neurological conditions, toxins or 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)

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Annette Karmiloff-Smith did her PhD in Psychologie Expérimentale et Génétique at Geneva University, Switzerland. After fellowships at UC-Berkeley, USA, and the Max Planck Institute, Nijmegen, Netherlands, she became an MRC Senior Scientist, 1982-1998, in London. From 1998-2005, she directed the Neurocognitive Development Unit, UCL Institute of Child Health, UK, and since 2005 is a Professorial Research Fellow at the Centre for Brain and Cognitive Development, Birkbeck, London UK. Her research focuses on tracing deficits in genetic disorders back to their neurocognitive roots in infancy. She is PI on the infant stream of the LonDownS Consortium.

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