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Down syndrome

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Abstract

Trisomy 21, the presence of a supernumerary chromosome 21, results in a collection of clinical features commonly known as Down syndrome (DS). DS is among the most genetically complex of the conditions that are compatible with human survival post-term, and the most frequent survivable autosomal aneuploidy. Mouse models of DS, involving trisomy of all or part of human chromosome 21 or orthologous mouse genomic regions, are providing valuable insights into the contribution of triplicated genes or groups of genes to the many clinical manifestations in DS. This endeavour is challenging, as there are >200 protein-coding genes on chromosome 21 and they can have direct and indirect effects on homeostasis in cells, tissues, organs and systems. Although this complexity poses formidable challenges to understanding the underlying molecular basis for each of the many clinical features of DS, it also provides opportunities for improving understanding of genetic mechanisms underlying the development and function of many cell types, tissues, organs and systems. Since the first description of trisomy 21, we have learned much about intellectual

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Author contributions

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disability and genetic risk factors for congenital heart disease. The lower occurrence of solid tumours in individuals with DS supports the identification of chromosome 21 genes that protect against cancer when overexpressed. The universal occurrence of the histopathology of Alzheimer disease and the high prevalence of dementia in DS are providing insights into the pathology and treatment of Alzheimer disease. Clinical trials to ameliorate intellectual disability in DS signal a new era in which therapeutic interventions based on knowledge of the molecular pathophysiology of DS can now be explored; these efforts provide reasonable hope for the future.

Down syndrome (DS) is the most common genomic disorder of intellectual disability and is caused by trisomy of *Homo sapiens* chromosome 21 (HSA21). The eponym of the syndrome is from Down, who described the clinical aspects of the syndrome in 1866 (REF.¹). The DS phenotype involves manifestations that affect multiple bodily systems, in particular the musculoskeletal, neurological and cardiovascular systems. Individuals with DS commonly have short stature, muscle hypotonia, atlantoaxial instability, reduced neuronal density, cerebellar hypoplasia, intellectual disability and congenital heart defects (CHDs; particularly atrioventricular septal defects (AVSDs)). Individuals with DS are also more likely to develop certain health conditions, including hypothyroidism, autoimmune diseases, obstructive sleep apnoea, epilepsy, hearing and vision problems, haematological disorders (including leukaemia), recurrent infections, anxiety disorders and early-onset Alzheimer disease (AD) (FIG. 1). Other conditions, such as most solid tumour types, show inverse comorbidity and seem to be less common in individuals with DS than in the general population².

The discovery of a link between a supernumerary chromosome 21 and the DS phenotype was first reported in 1959 (REF.³) and was an important landmark for the development of genetic medicine. Mouse models for the study of DS were first developed in 1990 (REF.⁴), and the complete nucleotide sequence of the long arm of HSA21 was published in 2000 by a multinational consortium of investigators⁵. Substantial progress has been made in the ensuing 19 years in understanding the molecular pathophysiology of the different phenotypic manifestations of DS, which is currently considered a disorder of gene expression dysregulation. In addition, widely used screening methods have been introduced for the prenatal detection of DS. The management of the different symptoms and the quality of life of individuals with DS have improved. However, enormous challenges remain, including understanding the precise biological mechanism of each phenotypic component of the syndrome; treatment of the different symptoms, including cognitive dysfunction; and integration of individuals with DS into society in different parts of the world. Furthermore, studies in animal models to unravel the effects of triplication of the >200 protein-coding genes on HSA21, not to mention the triplicated non-coding genes and the downstream and indirect effects of these alterations, are extremely challenging.

In this Primer, we discuss the epidemiology of DS, current understanding of genetics and pathophysiology resulting from the extra chromosome 21, and advances in the diagnosis of trisomy 21. Furthermore, we review the management of the syndrome and the quality of life of individuals with DS, and offer an outlook for the future. Several reviews of DS and

trisomy 21 have been published elsewhere and provide additional details about this common and challenging syndrome^{6–9}.

Epidemiology

Prevalence

The lifetime prevalence of DS is increasing substantially as the global population grows. For example, in the USA, the population prevalence of DS increased from ~50,000 in 1950 (3.3 per 10,000 individuals) to ~212,000 in 2013 (6.7 per 10,000 individuals; based on unpublished data¹⁰), mostly due to improvements in childhood survival of individuals with DS¹¹ (FIG. 2). The life expectancy of individuals with DS in the USA increased from an estimated mean of 26 years and median of 4 years in 1950 to 53 years and 58 years, respectively, in 2010 (REF.¹¹). As of 2015, estimates of DS population prevalence have been reported for Europe (4.9 per 10,000 individuals)¹², Europe excluding former Eastern Bloc countries (6.0 per 10,000 individuals), and former Eastern Bloc countries (3.3 per 10,000 individuals)¹². However, a precise global estimate cannot be reliably calculated until more birth registries are created within countries and more data are available on historical and current survival of individuals with DS in different parts of the world.

DS occurs in all populations, but differences in maternal age at conception between countries and ethnicities influence the number of live births^{13–16}. As of 2013 in the USA, unpublished data suggest that ~1 in every 779 babies born had DS¹⁰ (~12.8 per 10,000 live births) (FIG. 2a). The prevalence of DS is influenced by maternal age at conception, which varies among countries, and is estimated to be ~1 in 365 fetuses at 10 weeks gestation¹⁷ (FIG. 2b). Some of these pregnancies will result in spontaneous miscarriage, ~32% between 10 weeks gestation and the expected date of birth and ~25% between 16 weeks gestation and the expected date of birth, the risk depending on maternal age, as estimated from reports from England and Wales¹⁸. The number of pregnancies that are electively terminated is influenced by the availability and accuracy of screening tests within each country, the number of people choosing prenatal screening and subsequently prenatal testing, and parental decisions once a prenatal diagnosis of DS is made. In 2013, an estimated 3,400 DS-related elective terminations were carried out in the USA, resulting in a 33% reduction in the number of babies with DS that would have been born that year. By comparison, the estimated percentage reduction was 55% in Australia for 2004 (REF.¹⁹), and 54% in Europe, 66% in Europe excluding the former Eastern Bloc countries and 32% in former Eastern Bloc countries, for the period 2010–2015 (REF.¹²). In China, during the period 2003–2011, the termination rate for fetuses with DS led to a 55% reduction in the overall perinatal prevalence²⁰.

Studies from England and Wales²¹, Slovenia²², Australia^{19,23} and EUROCAT regions²⁴ have shown that increasing maternal age has counterbalanced the uptake in prenatal screening, resulting in a stable or slightly decreasing prevalence of DS in live births from the 1990s until now. In Europe, DS prevalence in live births has been decreasing slightly since the 1990s, although there are substantial regional differences¹². In southern Europe, DS prevalence in live births almost halved in the period 1980–2015 (REF.¹²). By contrast, in the Netherlands, the live birth prevalence increased slightly in the period 1980–2000 but seems

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to have been decreasing slightly since 2005 (REF.²⁵). In the USA, the increased prevalence of DS in live births began in the 1980s and levelled off after 2005 (REF.¹⁷). Sequencing cell-free placental DNA in maternal plasma, termed noninvasive prenatal screening (NIPS) or noninvasive prenatal testing (NIPT), has been available since 2011 in many countries; however, not enough time has elapsed for the full impact of surveillance programmes on birth rates to be measured. Furthermore, variability in access to prenatal care is also expected to affect the uptake of surveillance programmes.

Risk factors

Advanced maternal age at conception is a major risk factor for trisomy 21, as is true for all human autosomal trisomies²⁶. This risk is associated with non-disjunction of homologous chromosomes or chromatids occurring during the meiotic divisions that occur in the formation of oocytes²⁷. Advanced maternal age has been associated with HSA21 segregation errors in both maternal meiosis I and meiosis II^{28–32}. In addition, specific altered recombination patterns have been observed for these types of maternal errors, only some of which are associated with maternal age³³.

Meiotic processes, such as recombination, involve the action of many genes. Thus, there is a clear rationale for examining whether genetic variants predispose to meiotic nondisjunction in humans. A study including a candidate gene analysis and an untargeted genome-wide association study (GWAS) of HSA21 nondisjunction in mothers who gave birth to an infant with trisomy 21 derived from a maternal error was conducted using the mothers as cases and the fathers as controls³⁴. These analyses were stratified by maternal MI or MII errors. Results from this study emphasize the heterogeneity of risk factors based on type of error. For example, variants in candidate genes coding for components of the synaptonemal complex showed an association limited to meiosis I errors (for example, *SYCE2*) while others were associated with MII errors (for example, *SYCP2*)³⁴.

Environmental factors also influence the risk of non-disjunction but are difficult to identify due to the inherent problem of defining the exposure, dosage and timing of each factor. Again, studies must define the type of error (parental origin and the type of meiotic or mitotic error) leading to trisomy 21 due to their heterogeneous aetiology. Environmental factors that influence the risk of trisomy 21 include tobacco use, folic acid supplementation, oral contraceptive use and several others (the studies investigating environmental risk factors and their limitations, and possible biomarkers of exposure such as telomere length, have been reviewed elsewhere³⁵).

For example, maternal socioeconomic status (SES) is associated only with maternal meiosis II errors^{36–39}. As SES is a proxy for specific exposures, a follow-up study examined maternal occupation as a risk factor and found that some job classification categories were more prevalent in mothers of infants with DS than in mothers of infants with no chromosomal abnormality or major birth defect, and were associated with specific types of meiotic errors⁴⁰. A preliminary analysis revealed that these occupations involved exposure to solvents in the work environment. Further studies of this kind are needed to examine specific exposures to toxic agents in the work and home environments and their relationship with maternal and paternal HSA21 nondisjunction. This is emphasized by the finding that

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exposure to endocrine-disrupting chemicals affects meiosis and increases the prevalence of aneuploidy⁴¹. For example, exposure to the ubiquitous environmental contaminant bisphenol A (BPA) and other endocrine-disrupting chemicals affects the reproductive system in both sexes (including the ovaries, testes and reproductive tract)^{41,42}.

It is important to point out that not only do different types of meiotic and mitotic errors leading to aneuploidy most likely have different susceptibility to specific environmental exposures but that exposures of multiple generations (for example, grandmother and mother) must also be considered^{35,43}. For example, there is now mounting evidence of transgenerational effects of BPA exposure on sperm and oocytes in experimental models⁴².

Mechanisms/pathophysiology

Genetics of DS

Partial or complete trisomy 21 (that is, the presence of part of or a complete supernumerary HSA21) is the genomic cause of DS³. A free trisomy 21 is present in 95% of individuals with DS and results from an error in maternal meiosis I (~66%) or meiosis II (~21%); paternal meiosis I (~3%) or meiosis II (5%); or mitosis, after the formation of the zygote (5%)^{15,44,45}. Translocation accounts for trisomy 21 in ~5% of affected individuals, usually t(14;21) or t(21;21)^{46,47}. Mosaicism for trisomy 21 occurs in ~2% of individuals with DS. Partial trisomy 21 is rare^{48,49} and is associated with a range of symptoms that vary according to the length of the partial triplication of HSA21.

The first published sequence of HSA21 annotated 225 genes on chromosome 21q⁵. With increased knowledge of the elements of coding and non-coding genes and regulatory motifs, the number of gene structures recognized on HSA21 has increased considerably. The strengths and limitations of genome annotations available in existing public databases have been reviewed elsewhere⁵⁰. The current version of GENCODE/ENSEMBL (GENCODE release 32) lists 233 protein-coding genes, 423 non-protein-coding genes (69 small, 330 long and 24 miscellaneous non-coding genes) and 188 pseudogenes. Of note, 48% of HSA21 has not been annotated⁶, the vast majority of which contains repetitive elements (as is the case for all human chromosomes).

Understanding the genetic aetiology of the increased susceptibility of individuals with DS to the multiple manifestations or conditions that are associated with the DS phenotype is an enormous challenge⁵¹. Compounding the difficulty of identifying the specific genetic and other effects of trisomy 21, studies of DS in humans are restricted to those conceptuses that survive to term; among pregnancies with a confirmed prenatal diagnosis of DS at 9–14 weeks, ~30–40% subsequently spontaneously miscarry^{18,52}. Extrapolating to all pregnancies, ~80% of trisomy 21 conceptuses are lost during pregnancy⁵³. Thus, it is essential to acknowledge that in studying any DS-associated condition, only the combinations of genetic variants that allow the conceptus to survive to term and to manifest the phenotype of interest are being considered.

Two main hypotheses have been proposed to explain the biological perturbations that underlie the phenotypic manifestations of DS: first, a specific HSA21 gene-dosage

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effect, which includes both the direct effects of overexpressed HSA21 genes and the downstream consequences of this overexpression; and second, developmental instability, in which nonspecific global disturbance of gene expression from the extra HSA21 results in disruption of overall biological homeostasis^{54,55}. It is likely that the aetiology of DS-associated phenotypes involves both proposed mechanisms. Single-cell transcriptome analyses in trisomy 21 and other trisomies have suggested that the gene dosage effect for trisomic genes with low-to-average expression is mainly due to the higher fraction of trisomic cells expressing these genes⁵⁶. This results in the expected 1.5-fold higher average expression of trisomic genes in the various tissues of individuals with trisomy 21.

Gene dosage effects.—The simplest effect of HSA21 trisomy is the direct effect of an increased dosage of a single HSA21 gene. For example, an increased dosage of *APP*, an HSA21 gene that encodes amyloid precursor protein (APP), increases susceptibility to early-onset AD in individuals with DS. Although *APP* is clearly an ‘effector’ gene, whether only the direct and downstream effects of *APP* overexpression affect the penetrance and severity of AD, or whether other aspects of trisomy 21 also have a role, remains to be determined. Of note, returning the *App* copy number to two copies in a mouse model of trisomy 21 (Ts65Dn) alleviates some but not all of the effects of *App* dosage⁵⁷.

To define the direct and indirect consequences of increasing gene dosage, many studies have characterized the expression profile of HSA21 genes and the effect of overexpressing these genes on the rest of the genome. Common findings among these studies emerge. First, the expression of most but not all HSA21 genes is increased. It will be important to identify the mechanisms that underlie these discordant expression levels, which may include negative feedback, dosage compensation and epigenetic alterations. Second, in many affected individuals, the expression of some non-HSA21 genes is also altered, which suggests that trisomy 21 leads to perturbation of downstream transcription and signalling networks. A meta-analysis compared the expression profiles of trisomy 21 and euploid samples from various human tissues (such as brain and thymus) or cells (such as lymphoblastoid cell lines (LCLs), blood cells, fibroblasts and induced pluripotent stem cells (iPSCs))⁵⁸. This analysis found that the trisomy 21:euploid expression ratio was typically close to 3:2 for dysregulated genes and close to 1:1 for unaffected genes. These data are consistent with the hypothesis that upregulation of effector genes (that is, those that enhance or suppress gene expression) on HSA21 leads to similar changes in the expression of associated downstream genes, resulting in a similar altered ratio of 3:2 or 2:3 for these genes. There are exceptions to this pattern, with more extreme ratios that may result from gene–gene interactions or other types of chain effects that amplify the final expression levels. For example, *JAKMIP3* is highly upregulated (256.13:1 ratio) in the thymus transcriptome map, whereas *BEX5* is highly downregulated (0.07:1 ratio) in the brain transcriptome map.

Finally, as expected, the expression patterns of both HSA21 genes and non-HSA21 genes in trisomy 21 cells differ depending on the tissue being examined. For example, the interferon response is highly upregulated in trisomy 21 fibroblasts and LCLs, most likely due to increased expression of four interferon receptor genes (*IFNAR1*, *IFNAR2*, *INGR2* and *IL10RB*) that are located on HSA21 (REF⁵⁹). An increased interferon response was also

present in iPSCs generated from trisomy 21 fibroblasts but not in neuronal cultures derived from these iPSCs⁶⁰.

Disruption of homeostasis by genome-wide effects on transcription

regulation.—Trisomy 21 may also affect global transcription, either directly if an HSA21 gene functions in transcription regulation or indirectly as a by-product of the additional genetic material^{61,62}. For example, at least three HSA21 genes encode proteins involved in transcription regulation: ADARB1 (one of two proteins involved in adenosine-to-inosine RNA editing), the constitutive splicing factor U2AF1, and DYRK1A, a dual specificity kinase that phosphorylates splicing factors⁶⁰. Altered *ADARB1* transcript levels did not change global adenosine-to-inosine editing levels in trisomy 21 iPSC-derived neuronal cells, whereas trisomy 21-dependent splicing changes were observed in both iPSCs and the neuronal cultures derived from them⁶⁰.

The possibility that increased genetic material could lead to altered genome-wide gene expression was suggested based on the observation that genes whose expression is similarly altered (that is, upregulated or downregulated) are clustered in regions termed gene expression dysregulation domains (GEDDs) in trisomic iPSCs and fibroblasts⁶¹. However, other studies using a range of methods found no evidence for the existence of GEDDs specifically in trisomy 21 cells^{63,64}.

Studies of nuclear genome organization using three-dimensional fluorescence in situ hybridization (3D-FISH) have shown that chromosomes are preferentially localized to discrete regions within the nucleus, termed chromosome territories (reviewed elsewhere⁶⁵). Homeostasis in trisomic cells can be disrupted by the alteration of chromosome territories. Although studies of chromosome territories in trisomy 21 cells and their effects on cellular processes are just beginning, initial studies have found that an extra HSA21 does not change the overall organization of chromosome territories in the interphase nucleus⁶⁶ but does alter chromosome compaction and displaces other chromosome territories from their usual nuclear position⁶⁶.

Trisomy 21 also alters regional and/or global methylation patterns (reviewed elsewhere⁶²), although the methylation changes are evenly distributed on all chromosomes and are not specifically enriched on HSA21. One observed pattern is an overall bias towards hypermethylation in DS cells compared with euploid cells, particularly in brain samples^{67–69}. Furthermore, differential methylation in trisomy 21 cells seems to be localized to discrete regulatory regions of single genes and not to domain-like regions. A meta-analysis identified a small set of genes for which the methylation patterns were different between trisomy 21 and euploid cells in all tissues examined, suggesting that these altered methylation patterns are established early in development and thereby persist in multiple tissue types in DS⁶².

Two mechanisms have been proposed for the globally altered methylation patterns in trisomy 21. First, some triplicated HSA21 genes might function directly in methylation pathways, including *SLC19A1*, *FTCD*, *GART*, *CBS*, *PRMT2*, *N6AMT1*, *MIS18A* and *DNMT3L*. *SLC19A1*, *FTCD*, *GART* and *CBS* all have a role in the one-carbon

metabolism pathway, which is central to DNA methylation. *PRMT2* encodes a protein arginine methyltransferase with multiple targets, including histones⁷⁰. *N6AMT1* encodes a methyltransferase responsible for DNA N⁶-adenosine methylation⁷¹. The protein encoded by *MIS18A* is involved in maintaining the heterochromatic state of centromeres by recruiting the DNA methyltransferases DNMT3A and DNMT3B, thereby repressing the production of non-coding transcripts from centromeric satellite repeats⁷². DNMT3L also interacts with DNMT3A and DNMT3B and enhances their de novo methylation activity, but lacks DNA methyltransferase activity itself⁷³. *DNMT3L* is overexpressed in DS neuroprogenitors, which leads to increased expression of *APP* and *PSD95* in differentiating neurons⁷⁴. These examples illustrate the various mechanisms by which overexpression of these HSA21 genes may lead to trans-epigenetic changes in DS.

The second proposed mechanism is that differential methylation is a result of altered transcription factor occupancy of their binding sites. For example, RUNX1, an HSA21-encoded transcription factor, is overexpressed in trisomy 21 T lymphocytes and differentially methylated sites in trisomy 21 cells are enriched for the RUNX1 binding motif⁶⁷. Thus, the higher occupancy of RUNX1 binding sites in trisomy 21 cells affects CpG methylation at these sites. Additional support for this hypothesis comes from the observation that several types of sequence motifs, including the binding site for CTCF (an insulator protein that blocks the interaction between enhancers and promoters), are enriched at loci that are differentially methylated in all trisomy 21 tissues examined^{67,75}. Because CTCF binding to its recognition site is sensitive to methylation, the pattern of CTCF occupancy may be particularly affected by DS-associated epigenetic perturbations, which may result in altered 3D conformation of chromatin in the nucleus.

Histone deacetylation and acetylation are other epigenetic mechanisms that are potentially influenced by triplication of some HSA21 genes. For example, *DYRK1A*, which is overexpressed in trisomy 21, promotes histone deacetylation by phosphorylation and activation of the deacetylase SIRT1 (REF.⁷⁶). Furthermore, *DYRK1A* affects chromatin remodelling through its interaction with the transcription repressor REST (also known as NRSF)⁷⁷, resulting in neuronal gene dysregulation that might contribute to the neural phenotypic changes associated with DS.

Although genomic and transcriptomic data are important for understanding the consequences of trisomy 21 and how they relate to interindividual variation in clinical manifestations, integration with other ‘omics’ datasets is necessary to obtain a complete picture of the effects of trisomy 21. For example, in a proteomic study⁷⁸ using a method that analysed a large fraction of the proteome⁷⁹ of fibroblasts from a pair of monozygotic twins discordant for DS (with follow up on samples from 11 unrelated individuals with DS and matched controls), extensive changes were detected in the levels of proteins encoded by HSA21 genes and non-HSA21 genes. In transcriptome data from both of these twins⁶¹, steady-state transcript levels were moderately correlated with protein levels. However, the trisomy 21:euploid ratios for gene expression and protein levels were only weakly correlated (Spearman rank correlation $\rho = 0.34\text{--}0.51$), which was mostly due to incongruence of transcript and protein levels for non-HSA21 genes. Thus, substantial post-transcriptional regulation has a role in the differential effect of trisomy 21 on the

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expression levels of different genes. For example, for HSA21-encoded proteins that are components of heteromeric protein complexes with non-HSA21-encoded proteins, protein degradation seems to buffer against increased transcript levels. Gene enrichment set analyses have shown that protein levels are substantially altered for cell cycle-related functions, cell morphogenesis, lipoprotein metabolism and cellular respiration in mitochondria⁷⁸.

In another study⁸⁰ using a proteomics method focused primarily on secreted proteins and those with extracellular domains⁸¹, plasma samples from 165 individuals with DS and 98 euploid individuals were compared. Among the proteins that were dysregulated in trisomy 21 samples, many are involved in immune control, the complement cascade and growth factor signalling. However, no clear link exists between the identity or levels of dysregulated proteins and specific clinical manifestations of DS.

Indicators of mitochondrial dysfunction have been observed in trisomy 21 cells (for example, fibroblasts) and organs, including the heart (their properties and consequences have been reviewed elsewhere^{82–84}). The mitochondrial phenotype in DS includes reduced ATP production by oxidative phosphorylation; decreased respiratory capacity; impaired generation of mitochondrial membrane potential; and alterations in mitochondrial structure. These altered functions result in perturbed mitochondrial energy metabolism and oxidative stress, which in turn could increase susceptibility of individuals with DS to a wide range of conditions, including intellectual disability and AD. The molecular basis for mitochondrial dysfunction involves effector HSA21 genes and key regulatory signalling pathways (reviewed elsewhere⁸²). Of 77 HSA21 genes consistently dysregulated identified by a meta-analysis of 45 DS gene expression studies⁸⁵, *NRIP1*, *SUMO3*, *DYRK1A*, *RCAN1*, *SOD1*, *APP* and *CBS* are directly or indirectly involved in mitochondrial function and thus represent candidates for the observed DS-associated mitochondrial phenotypes.

Animal models

Animal models of DS, especially mouse models, have been instrumental in advancing DS research. However, a caveat when studying these models is that DS is a human condition that cannot be precisely replicated in other species. Nonetheless, insights into the mechanisms that underlie the developmental and functional consequences of trisomy 21 have been obtained by overexpression of groups of HSA21 genes or orthologues of HSA21 genes from other species. Important questions about the usefulness of animal models for studying trisomy 21 pathophysiology include the question as to what types of studies are informative in mice, whether results in mice are relevant to understanding human outcomes of trisomy 21 and whether mouse models of DS can be used as a translational platform for drug testing.

Mouse models have transformed basic research in DS, beginning with development of the Ts65Dn mouse, which contains a partial trisomy of *Mus musculus* chromosome 16 (MMU16)^{4,86}. Prior to the introduction of this mouse model, the identification, cloning and expression of individual genes in transgenic mouse models was laborious, and studies using these approaches led to wide-ranging conclusions about individual genes that could ‘cause’ DS. However, overexpression of dozens or hundreds of genes simultaneously, supported by

more precisely defined terminal phenotypes, has enabled a more realistic interpretation of studies of development, function and genome-wide perturbations in gene expression.

To understand the challenges of using mouse models, it is necessary to understand the complexity of trisomy 21. Trisomy 21 has profound phenotypic effects, as HSA21 contains >200 protein-coding genes and ~400 additional transcripts of known or presumed importance, which are present in an extra copy in every cell from conception onwards. Conversely, the gene overexpression changes in trisomy 21 are subtle, with an average increase in expression of ~1.5-fold in trisomic cells compared with euploid cells. Expression levels of individual genes vary widely and mRNA levels do not always correlate with the levels of the corresponding protein⁷⁸. For genes expressed at a low level, this difference cannot be reliably measured with precision in many high-throughput assays. The consequences of this modest overexpression are substantial, as up to 80% of conceptuses with trisomy 21 do not survive to term¹⁵.

Relevance of mouse phenotypes to DS.—Several phenotypic features of DS manifest (to varying extents) in all individuals with trisomy 21, including cerebellar hypoplasia, retrusion of the midface skeleton and mandible, Alzheimer-disease-type histopathology at an early age, and intellectual impairment. The risk of CHDs is greatly increased in individuals with DS. Whereas adults with DS show an overall lower risk of developing solid tumour cancers, including breast cancers, when compared to the general population, children with DS have an increased incidence of leukaemia². All of these features are present in some form in the animal models of DS that have been generated to date. For example, whereas the overt histopathology of AD (plaques and tangles) does not occur in mouse models of trisomy 21, accumulation of tau protein and endosomal changes have been reported in these mice⁵¹.

An overall finding from animal models of DS is that the penetrance (occurrence) and expressivity (severity) of a given phenotype is a function of overexpression of multiple genes; DS is not a collection of independent single gene effects. However, many mouse models that are trisomic for different subsets of mouse orthologues of HSA21 genes have been used effectively — especially in combination with individual gene knockouts that allow specific genes to be returned to the normal two copies in a trisomic background — to identify genes with major effects that are important for understanding a number of phenotypes^{57,87,88}. Rather than asking the simplistic question of whether a gene causes DS, different mouse models provide information about whether altered expression of a specific gene or genes is necessary and/or sufficient to contribute to a phenotype. Interpretation is then limited by the ability to quantify the effects of trisomy in an animal model. Saying that a given model has incompletely penetrant CHDs is of little use in understanding the contributions of specific genes to specific outcomes; failure of ventricular or atrial septum development, AVSDs or outflow tract anomalies all occur in different developmental fields involving different cells at different stages of heart development with the contribution of different genes.

Genetic basis of animal models.—The extent of the triplicated region of HSA21 or the orthologous mouse genomic regions (that is, a segment of a mouse chromosome)

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is an important consideration in determining the relevance of a mouse model of DS. Trisomy for mouse genomic regions orthologous to HSA21 has the advantage that these regions are subject to endogenous transcription regulation, whereas genetic variation in, for example, promoter or enhancer sequences between mouse and human might be expected to affect transcription. Similarly, any normal human-specific variation in processing signals in DNA and RNA, or amino acid substitutions in proteins, could affect the stoichiometry of interactions between HSA21-encoded proteins and non-HSA21-encoded proteins. Data from GWAS and RNA sequencing suggest that these effects are small, but their potential importance cannot be dismissed. Conversely, although the gene content of HSA21 and the orthologous segments on MMU16, MMU17 and MMU10 are similar, they are not identical (FIG. 3). Of the 233 predicted protein-coding genes on HSA21, only 168 are well conserved in mice. Furthermore, the difference in number and sequence between human and mouse in the non-coding transcripts of presumed function is even greater. The limitations of individual mouse models are emphasized in a comparative study of three of these models, Ts65Dn, Ts1Cje and Ts1Yey⁸⁹.

These differences have not been emphasized in the past, as only one mouse model (Tc1) that contained a freely segregating HSA21 existed. To date, Ts65Dn is the only reported mouse model of DS that segregates an extra mouse chromosome; all other models are made by direct duplication of a mouse chromosome segment that is orthologous to HSA21 (detailed mapping of the partial mouse trisomies of segments orthologous to HSA21 has been reviewed elsewhere^{6,7}). Genome sequencing in Tc1 mice has revealed substantial deletions, mutations and duplications of HSA21 that compromise the expression of >20% of HSA21 genes in these mice⁹⁰. However, the biggest drawback of Tc1 is the spontaneous loss of the entire HSA21 in a substantial proportion of cells, resulting in mosaic trisomy (that is, each animal is a unique mosaic of trisomic and euploid cells). As development is driven and regulated through cell–cell communication, many aspects of an individual are liable to vary if neighbouring cells suddenly change ploidy. As the loss of HSA21 is random, the development of every animal is unique. Consequently, these rearrangements and mosaicism necessitate cautious interpretation of studies using Tc1 mice. A new model containing 92% of the protein-coding genes on the long arm of HSA21 as a mouse artificial chromosome avoids the problem of mosaicism and has many of the DS manifestations of trisomy 21 that have been observed in previous mouse models⁹¹.

The demonstration that directed interchromosomal recombination could be achieved using the Cre–Lox system paved the way for the creation of new mouse models for dosage imbalance studies^{92,93}. To date, >20 mouse models that are trisomic for different HSA21-homologous portions of MMU16, MMU17 and MMU10 have been created⁷ (FIG. 3). A set of three mouse strains that together contain a complete duplication of all mouse genomic regions orthologous to HSA21 has been described⁹⁴. In theory, a three-way cross of these mouse strains should produce ‘full trisomy’ in one out of eight offspring. Although this low frequency is only acceptable for some applications, in practice, the frequency of full trisomy is far lower than the predicted Mendelian frequencies, making models such as Tc1 or the MAC21 mice the most viable options for maximum gene representation.

Whereas 95% of individuals with DS have an extra freely segregating HSA21 and therefore an extra centromere and telomeres, the segmental duplication mouse models of DS do not. In these models, trisomy occurs through direct intrachromosomal duplications so that chromosome (and centromere) number is unchanged. One effect of an extra centromere in mammals is reduced male fertility, which is observed in human males with DS and in mouse models of DS. The exact mechanism of this is not well understood⁹⁵. Furthermore, trisomic cells seem to proliferate more slowly and/or have a prolonged cell cycle compared with euploid cells^{96,97}. Although the results of many of these studies are contradictory, it is feasible that very small changes in the cell cycle could have a major effect on development. As human development requires 43 population doublings to progress from a single cell to the estimated one trillion cells in a fetus (if all of the cells survived — the actual number is clearly much higher), a very small increase in cell cycle length could result in recognizable phenotypes, such as small stature, short fingers, skeletal retrusion, decreased neuronal density in the brain and hypocellular tissues.

Pathophysiological insights from animal models.—In bio-medical research, mouse models provide an important translational tool to identify and prioritize therapeutic approaches to ameliorate the effects of a condition. Efforts to correlate DS-like phenotypes in mice with trisomy of specific genes (and groups of genes) have led to the development of more than 20 mouse models with partial trisomy⁷ (FIG. 3). Although the mouse provides a good mammalian genetic model for understanding DS pathogenetic mechanisms, it is important to keep in mind that mice do not develop DS. However, mouse models do illustrate several fundamental principles of gene effects in trisomy and preclinical pharmacological amelioration seems promising, especially for cognitive effects. Trisomic genes that contribute to a deleterious phenotype are targets for drug development to ameliorate features of DS. To illustrate the application of mouse models, examples of the use of mouse models to identify the genetic basis of trisomy effects in brain function, heart development and cancer risk are discussed.

Many preclinical studies emphasize improving cognitive function, possibly the most difficult target for pharmacological intervention. Ts65Dn and many subsequent models exhibit learning and memory deficits and electrophysiological changes consistent with altered synaptic function in the hippocampus^{86,98,99}. Interestingly, Ts65Dn mice showed a significant learning deficit compared with Ts1Cje mice in a standard memory test⁵⁷. Ts65Dn is trisomic for a larger number of genes than Ts1Cje (FIG. 3), one of which is *APP*. Returning *APP* copy number to two by crossing a null allele of *APP* into the Ts65Dn background substantially improved the performance of these mice, although not to the level of a euploid mouse.

CHDs are present in ~50% of babies born with DS and mouse models display some analogous developmental deficits, albeit at a lower frequency than in human babies¹⁰⁰. To identify a gene or genes that cause CHDs when present in three copies, seven (partial or complete) trisomies of the ~23 Mb region of MMU16 orthologous to HSA21 were studied¹⁰¹, which narrowed down the region responsible for causing CHDs (especially septal defects reminiscent of those in DS) to a 4.9 Mb portion of MMU16. However, three smaller trisomies subdividing the 4.9 MB segment did not have heart defects, suggesting that at least

two genes must be trisomic to cause CHDs. In a different approach, interactions between trisomy and disomic modifiers of CHD risk were examined. A mouse hemizygous for *Creld1* (a gene implicated in familial AVSD) was crossed with Ts65Dn and Ts1Cje mice¹⁰². Whereas *Creld1*^{+/−} mice had normal heart development, the occurrence of septal defects was markedly increased in *Creld1*^{+/−} Ts65Dn mice but not in *Creld1*^{+/−} Ts1Cje mice. Of the genes that are trisomic in Ts65Dn but not in Ts1Cje, 14 are expressed in heart development, including junctional adhesion molecule 2 (*Jam2*), which consistently increases the frequency of heart abnormalities when overexpressed in zebrafish¹⁰³. Restoration of two copies of *Jam2* in *Creld1*^{+/−} Ts65Dn mice blocked the increased occurrence of CHD in these mice. Thus, trisomy for *Jam2* is required for the disomic risk factor gene *Creld1* to affect heart development.

Animal models have also been important in understanding cancer risk in individuals with DS. Epidemiological studies provide strong evidence that the incidence of many cancers is reduced in adults with DS¹⁰⁴. Importantly, studies in mouse models of DS have confirmed these results, showing that partial trisomy 21 protects against various cancers. For example, familial adenomatous polyposis (FAP) is a congenital condition involving the formation of precancerous adenomatous polyps (predominantly in the colon) at an early age, and is caused by mutations in the tumour suppressor adenomatous polyposis coli (*APC*). Interestingly, Ts65Dn mice carrying an *Apc* mutation found in patients with FAP (*Apc*^{Min}) develop 50% fewer adenomatous polyps than euploid mice⁸⁷. Ts1Rhr mice, which are trisomic for just 33 of the genes triplicated in Ts65Dn, have a similar reduction in adenomatous polyp formation. This reduction is substantially reversed by restoring just one of these 33 genes, the proto-oncogene *Ets2*, to two copies. Furthermore, *Apc*^{Min} mice monosomic for these 33 genes (including *Ets2*) develop more tumours than euploid *Apc*^{Min} mice. Thus, assessment in mouse models of trisomy and monosomy has uncovered a protective effect of *Ets2* overexpression and a tumorigenesis-permissive effect of reduced *Ets2* expression.

Preclinical studies and drug development.—Several clinical trials of pharmaceutical and nutraceutical agents to improve cognition in individuals with DS were initiated on the basis of preclinical observations in mouse models of DS (for example, [NCT00748007](#) for rivastigmine, [NCT02484703](#) and [NCT02024789](#) for RG1662, and [NCT01112683](#) for memantine). Although in-depth discussion of all of these trials is beyond the scope of this Primer, for illustrative purposes we discuss preclinical studies that led to phase I and II trials of RG1662 (also known as basmisanil or RO5186582), an inverse agonist of GABA_A α5 receptors. Cognitive tests in Ts65Dn mice showed that these mice have deficits in learning and memory in tasks that depend on hippocampal function^{86,105,106}, prompting electrophysiological studies demonstrating that long-term potentiation (LTP) is reduced in hippocampal slices from Ts65Dn mice^{98,99,107}. The reduced LTP stemmed from excess GABA-mediated inhibition, which could be reversed by treatment with the GABA_A inhibitor pentylenetetrazol (PTZ), restoring the inhibitory–excitatory balance and thereby improving performance in cognitive tests, even in young adult mice¹⁰⁸.

These studies profoundly changed the understanding of cognitive dysfunction in DS and its treatment, by showing not only that treatment of cognitive deficits in DS is possible but that

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it is effective in adults, when previously it was widely thought that cognitive improvements could only be made during a small window early in life. Furthermore, this treatment had long-lasting effects, as the improved LTP was measured 3 months after cessation of PTZ treatment¹⁰⁸. Thus, treatment for cognitive deficits is not a nebulous goal far in the future but can and should be addressed immediately. An inverse agonist of GABA_A α5 receptors restores LTP and improves performance in cognitive tests in Ts65Dn mice^{109,110}. RG1662 was assessed for safety and tolerability in individuals with DS in a successful phase I trial ([NCT01436955](#)) that concluded in 2013. However, a phase II trial begun in 2014 was stopped prematurely in 2016 because RG1662 did not show efficacy.

Although the lack of efficacy in this specific trial design was disappointing, the trial represented several advances. First, it demonstrated that the pharmaceutical industry now had the confidence to treat intellectual disability, a first for the DS community. Second, the ability to enlist multiple centres throughout the USA and Europe that were capable of the complex measurements used in the trials, combined with strong support from the DS community in recruiting individuals for the trials, was a clear statement of interest by researchers, clinicians and individuals with DS. Finally, the application of cognitive tests designed specifically for DS was an important part of the trial. Although the official summary of results from this trial have not yet been published, the principles involved in treating individuals with DS have been reported by a number of the investigators involved in the trial¹¹¹. Overall, the disappointment of the trial result was overlaid with optimism for improved treatment and trial design approaches in the future, and informed further trials such as a recent phase II trial using green tea extract containing epigallocatechin-3-gallate (EGCG) to improve cognitive outcomes¹¹².

Mechanisms of Alzheimer disease

Almost all adults with DS develop AD-like neuropathology by 40 years of age. AD is characterized by a long asymptomatic preclinical stage in which amyloid pathology develops, ~15–20 years before any cognitive impairment is observed¹¹³. This preclinical stage is present in autosomal dominant forms of AD¹¹⁴ and in early-onset AD associated with DS¹¹⁵. The evidence is compelling that an increased dosage of *APP* in trisomy 21 is necessary for increased risk of AD in individuals with DS, although the underlying mechanisms that link *APP* dosage to neurodegeneration are unknown. However, at least three molecular mechanisms have been proposed to explain the increased risk of AD in individuals with DS (FIG. 4).

APP and Aβ42.—Increased *APP* expression resulting from the presence of an additional copy of the *APP* gene is strongly correlated with early-onset AD^{116,117}. Genomic duplication of the *APP* locus is the cause of some cases of familial, early-onset AD^{118,119}. In individuals with DS with complete HSA21 trisomy, the extra copy of HSA21-located *APP* results in increased levels of APP and its cleavage products, including the neurotoxic Aβ42 peptide, in the brain¹²⁰. However, individuals with partial trisomy of HSA21 who are disomic for APP do not develop early-onset AD and have normal APP expression levels¹²¹.

DYRK1A.—A second mechanism involves multiple neuropathological effects of *DYRK1A* overexpression. HSA21-encoded DYRK1A phosphorylates APP at Thr668, resulting in increases phosphoAPP levels in cells from a mouse model of DS that overexpresses the human *DYRK1A* gene¹²². This phosphorylation facilitates the amyloidogenic processing of APP by β-secretase and γ-secretase, leading to increased production of neurotoxic Aβ42 peptide^{123,124}. The levels of phosphoAPP and Aβ42 are increased in the brain of these mice, and phosphoAPP, DYRK1A and Aβ42 levels are increased in the brain of individuals with DS.

DYRK1A also has effects on another molecule implicated in the pathophysiology of AD, the microtubule-stabilizing protein tau. DYRK1A phosphorylates tau at Thr212, a residue that is hyperphosphorylated in individuals with AD¹²⁵. Abnormal hyperphosphorylation of tau changes its conformation, leading to a reduced affinity for microtubules¹²⁶ and resulting in microtubule instability, neurofibrillary tangle (NFT) formation and cell death¹²⁷. Furthermore, DYRK1A also phosphorylates splicing factors and thereby alters the splicing of the mRNA of the tau-encoding gene *MAPT*. Alternative splicing of *MAPT* results in the production of tau protein isoforms with three or four microtubule-binding domains (three-repeat (3R) tau and 4R tau, respectively). Equal levels of 3R tau and 4R tau are required for maintaining normal brain function and an imbalance in their levels has been detected in sporadic tauopathies¹²⁸. DYRK1A overexpression increases the abundance of 3R tau at the expense of 4R tau, which is thought to lead to the formation of NFTs, as 3R tau has lower affinity for microtubules than 4R tau^{129,130}.

Endosomal dysfunction.—An extra copy of *APP* is sufficient to cause endosomal enlargement and intracellular trafficking defects by an Aβ-independent mechanism¹³¹. RAB5 mediates the endocytosis of cell-surface proteins and the homotypic fusion of early endosomes¹³². Enlargement of RAB5-positive early endosomes, a phenotype consistent with excessive activation of RAB5 (REF.¹³³), is present in neurons in the brain of individuals with sporadic or familial AD^{134,135} and in individuals with DS who develop AD¹³⁶. In fact, endosomal size in circulating peripheral blood mononuclear cells might serve as a blood-based biomarker of AD-related endosomal pathology¹³⁷.

Endosomal function is also affected by other factors in DS. The ε4 allele of *APOE* (*APOE**ε4) is the most important genetic risk factor for late-onset AD, as ~80% of all patients with AD have at least one *APOE**ε4 allele¹³⁸. *APOE**ε4 is also thought to increase the risk of dementia in older adults with DS, albeit to a lower extent than for sporadic AD in euploid individuals¹³⁹. Endosomal enlargement in trisomic neurons is thought to cause axonal trafficking defects that contribute to neuronal degeneration¹⁴⁰. Furthermore, *APOE* regulates the endosomal trafficking of amyloid fibrils¹⁴¹ and early endosomal enlargement is present in the brain of mice carrying *APOE**ε4 (REF.¹⁴²). *APP* gene dose, Aβ42 and the C-terminal fragment of APP, C99 (also known as βCTF), also have important roles in the development of endosome dysfunction. An increased level of C99 is the only APP-related alteration associated with abnormal endosome enlargement and proliferation that is common to all forms of AD^{143–146}.

As *APP* gene dosage is an important determinant of AD risk in individuals with DS, various therapies to reduce *APP* levels in individuals with DS might have efficacy in treating AD, including reducing *APP* expression (using anti-sense approaches) and *APP* production (using translational inhibitors such as Posiphen), blocking *APP* cleavage (such as by BACE or DYRK1A inhibition or using γ -secretase modulators) or removing A β (by active or passive A β immunization).

Diagnosis, screening and prevention

Prenatal screening

In developed countries, laboratory-based prenatal screening for DS is offered as part of routine antenatal care. Screening is a way to identify pregnancies at high risk, thereby limiting diagnostic procedures to minimize the risk of an iatrogenic miscarriage. Since the 1980s, the primary prenatal screening approach has involved a combination of measuring maternal serum biochemical analytes and, more recently, the size of the fetal nuchal translucency (NT; a pouch of fluid behind the neck) in the first trimester¹⁴⁷. Initially, levels of α -fetoprotein in maternal serum and amniotic fluid in the second trimester were measured and a level ~70% of that in a normal pregnancy indicated an elevated risk of a DS conceptus. More recently, additional maternal serum analytes have been measured, including β -human chorionic gonado-tropin, unconjugated oestriol, inhibin A and pregnancy-associated plasma protein A. The levels of all of these markers change with gestational age, and some are better at distinguishing between trisomy 21 and euploid fetuses in the first trimester and others in the second trimester. Thus, correct dating of the pregnancy by ultrasonography examination is needed for accurate interpretation of serum analyte test results. For each woman, the risk of DS in the fetus is calculated using a computer algorithm with inputs of raw analyte values, gestational age and demographic information, such as maternal age, geo-ethnic background, smoking status and whether or not she has diabetes. The numerical risk cut-off values used in clinical practice differ in their reference points (that is, the risk of an affected fetus versus a liveborn infant). The chance of having a liveborn infant with DS is lower than the chance of having a fetus with DS in the second trimester because some of the fetuses will spontaneously miscarry in the second trimester¹⁴⁸. Professional guidelines recommend that pregnant women with positive screening results are offered post-test counselling and a diagnostic test, such as amniocentesis or chorionic villus sampling followed by genetic analysis.

In the late 1980s and early 1990s, prenatal ultrasonography was incorporated into routine care, and some abnormalities were demonstrated to be associated with DS. Of note, no fetal anatomical findings are diagnostic of DS and, in fact, many neonates with DS have apparently normal prenatal sonograms. First-trimester ultrasonography features that may indicate DS include an increased NT measurement for gestational age and four other first trimester markers: absent nasal bone, increased frontomaxillary angle, tricuspid valve regurgitation and absent or reduced flow in the ductus venosus¹⁴⁷. A second trimester anomaly scan has become routine at 18–20 weeks of gestation and includes quantifiable markers, such as a thickened nuchal skin fold and femoral and humeral length measurements. Additional so-called ‘soft’ ultrasonography markers include cystic hygroma,

prominent tongue, choroid plexus cysts, mild ventriculomegaly, heart defects, echogenic bowel, duodenal atresia, pyelectasis, bilateral fifth finger clinodactyly and a wide space between the great and second toes¹⁴⁸.

Using a combination of either first or second trimester maternal serum analyte quantification and NT measurement, the positive predictive values (PPV) for positive screening results are ~3–5% (refs^{149,150}). Because of these low PPVs, there has been longstanding interest in a more precise NIPS for fetal chromosome aneuploidies. In 2011, sequencing of cell-free DNA in maternal serum became clinically available¹⁵¹. Using massively parallel sequencing, the DNA fragments are counted, mapped to specific areas of the genome, and compared to a reference value; an excess number of fragments that map to HSA21 is suggestive (but not diagnostic) of DS¹⁵¹. Within a very short time, NIPS has become the most clinically implemented example of genomic medicine, with ~10 million tests performed to date¹⁵². Currently, in the USA, a pregnant woman at high risk of having a fetus with DS (maternal age >35 years, positive family history, positive serum analyte and/or NT measurements, suggestive ultrasonographic abnormalities) is offered NIPS as a primary screen. In many countries in Europe, NIPS is beginning to be offered as a secondary test following a positive primary screen. At present, only in Belgium and the Netherlands is NIPS offered as a primary screen for all pregnant women, regardless of a priori risk.

A meta-analysis examined the PPV of NIPS in pregnant women at high or low risk of having a fetus with DS¹⁵³. For DS, the PPVs were 91% and 82% for high-risk and low-risk pregnant women, respectively. Studies that directly compared the predictive performance of analyte and/or NT measurement to that of NIPS in the same pregnant woman have demonstrated a 10–20-fold increase in PPVs using sequencing^{149,150}. Importantly, NIPS is not dependent on gestational age and can be performed at any time between 10 weeks of gestation and delivery. Because NIPS performs better than current standards of care, in some countries with nationalized health systems there is a move towards providing NIPS as a primary screen for DS¹⁵¹.

Because NIPS is noninvasive and has a very good PPV, there have been concerns about what effect this testing will have on the live birth rates of infants with DS. A preliminary study found no difference in the number of live births of babies with DS before and after NIPS became available in eight countries¹⁵⁴. However, without access to all prenatal testing results, it is unclear how many more babies with DS would have been born during this time period. More extensive studies are needed to assess the effect of NIPS on the incidence of DS. Although many women choose not to have prenatal testing because it would not influence their reproductive decisions, in one study, women who knew prenatally that their babies had DS had better results on psychological tests than women who discovered at birth that their baby had DS¹⁵⁵. A prenatal diagnosis of DS has been reported to have several benefits, including opportunity for parental education, meetings with paediatric subspecialists ahead of delivery, and change of the location of delivery so that qualified paediatric subspecialists are available for care, thereby enabling mother and baby to remain in the same hospital together¹⁵⁵. A future benefit of accurate prenatal screening could include the opportunity to initiate treatment during fetal life to improve cognition (discussed later).

Management

Each individual with DS has a distinct set of strengths and challenges that can change throughout his or her life¹⁵⁶. Some individuals will require high levels of medical input from birth, whereas others may have few health complications. Similarly, some individuals will require social care and support throughout adulthood, whereas others live independently. Several health problems are more common in individuals with DS than in the general population, including CHDs, obstructive sleep apnoea, thyroid disease, dementia, epilepsy, gastrointestinal disease, hearing and vision problems, intellectual disability and developmental disorders, mental illness, immunological dysfunction, haematological disorders and musculoskeletal issues. Screening for these manifestations should be carried out regularly and there are consensus screening guidelines for children with DS (such as the guidelines of the American Academy of Pediatrics¹⁵⁷) but not yet for adults^{158–160}. Services for adults are often more specialized than those for children, and care usually needs to be managed by a number of different medical teams. No consensus currently exists about who should oversee care in adults with DS, but primary care physicians often take on this role¹⁰⁰; occasionally, paediatricians may remain involved until early adulthood, and in some countries, such as the UK, intellectual disability psychiatrists are most likely to provide medical support within community teams. Due to the lack of consensus, adults with DS may miss out on regular screening and proactive treatments, with interventions only occurring when difficulties are clinically apparent¹⁶¹.

Illness and disease may present differently in people with DS. There is a risk of diagnostic overshadowing and misattribution of symptoms, which can be compounded by the high prevalence of communication difficulties in individuals with DS. Due to the multisystem involvement in DS, care requires multidisciplinary input from a range of medical, social care and educational teams.

Perinatal management

Pregnant women carrying a fetus with a confirmed DS diagnosis require regular monitoring and support throughout the perinatal period. Pregnant women carrying a fetus with DS are at increased risk of miscarriage, with an estimated rate of spontaneous fetal death after 12 weeks of 30%, which increases with maternal age¹⁸. Monitoring recommendations suggest that a detailed ultrasonography examination and fetal echocardiography should be performed at 18–20 weeks gestation, with further ultrasonography examinations at 28–30, 34–36 and 38 weeks to assess for evidence of upper gastrointestinal obstruction, chylothorax, fetal hydrops and intra-uterine growth restriction. If abnormalities are detected, then increased fetal surveillance is recommended. Local paediatric teams should be informed if abnormalities are detected so that they can be involved in planning postnatal care and treatment (for guidance see, for example, the Down's Syndrome Association and the Down Syndrome Medical Interest Group fact sheet).

Congenital heart defects

CHDs occur in ~50% of individuals with DS, most commonly AVSD (42% of CHDs in individuals with DS), ventricular septal defect (22%) and atrial septal defect (16%)¹⁶².

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Although the frequency of the specific type of CHD depends on age and ethnicity, the primary point is that CHDs have a severe effect on the quality of life of the individual. During pregnancy, a fetal echocardiography examination is recommended. A cardiology examination should take place postnatally, and another echocardiography examination should be performed within the first month after birth. Management is the same as for the general population, including surgical repair¹⁶³. The mortality rate after surgery in children with DS is equal to or lower than that in the general population¹⁶⁴. All individuals with DS should have annual screening throughout life for signs of acquired valve disease and heart failure.

Sleep apnoea

Obstructive sleep apnoea is common in individuals with DS, with an estimated prevalence of 54–90%^{159,165}. Screening for symptoms should be carried out at every health check; these symptoms include loud snoring, heavy breathing, restless nights and daytime sleepiness, as well as neurocognitive symptoms, such as irritability, depression, paranoia, cognitive decline and behavioural problems¹⁶⁶. Overnight polysomnography is recommended for all children with DS before 4 years of age, regardless of symptoms^{157,167}. Alternative approaches, such as home oximetry, have been suggested to enable identification of at-risk individuals and to reduce the number of children that require full diagnostic studies¹⁶⁸. Management of sleep apnoea includes the use of continuous positive airway pressure (CPAP), mandibular advancement devices and weight loss. Surgical interventions, including tonsillectomy and adenoidectomy, can be considered, although sleep apnoea may persist after surgery^{165,169}.

Thyroid dysfunction

Congenital hypothyroidism is present in ~1% of individuals with DS, and abnormal thyroid function tests have been reported in >50% of neonates with DS^{170–172}. An increased risk of developing thyroid disease remains throughout life, and the risk of developing autoimmune-related thyroid dysfunction increases with age¹⁷³. As clinical diagnosis can be difficult, it is important to perform regular blood screening. Thyroid-stimulating hormone (TSH) and thyroxine (T4) levels should be obtained postnatally and at 6 months and 12 months of age. Measurements of TSH should then be repeated annually¹⁵⁷.

Alzheimer disease

A substantial proportion of individuals with DS develop early-onset AD, which is related to APP overproduction^{120,174}, and dementia is the proximal cause of death in 70% of older adults with DS¹⁷⁵. Clinical symptoms appear after 40 years of age, and 77% of individuals with DS aged 60–69 years, and up to 100% of those aged >70 years, will develop cognitive decline^{176–178} (FIG. 5). The cognitive decline needs to be understood in the context of the existing cognitive phenotype and individual risk, including *APOE* genotype, as the presence of the *APOE* ε4 allele seems to increase the risk of earlier cognitive decline due to AD and of mortality¹⁷⁹ (FIG. 5b).

Deficits in memory and attention occur early in DS, as in individuals with sporadic AD¹⁸⁰, but often go unnoticed until behavioural changes appear¹⁸¹. Some individuals with DS and

dementia may present with seizures and, as the disease progresses, the development of neurological symptoms, such as myoclonus and seizures, is commonly seen¹⁸².

A baseline assessment of cognitive and adaptive functioning at 30 years of age is recommended in all individuals with DS, to aid future monitoring and diagnosis¹⁸³. Management should focus on early detection and supportive measures. Positive benefit may be obtained from the use of acetylcholinesterase inhibitors^{184,185}, although some individuals may develop a reduced heart rate and other adverse effects. Individuals with DS and dementia should have access to adequate support, as their needs will increase as the disease progresses. Efforts should be made to keep individuals within their familiar homes, and transfer to other providers should be based on individual circumstances and needs¹⁸⁶.

Epilepsy

Epilepsy is present in 8% of children with DS, with a bimodal age of onset (one peak before 3 years of age and the other after 30 years of age¹⁶⁰). Infant onset, most frequently infantile spasms, has been associated with a severe form of epilepsy termed West syndrome, which includes psychomotor regression and hypsarrhythmia (an abnormal pattern of activity between convulsions) on electroencephalography, and occurs in ~6–32% of seizures reported in infants¹⁸⁷. Later onset of epilepsy is linked to the development of AD¹³⁶. The management of epilepsy depends on the aetiology, but usually involves anticonvulsant medication, which is generally effective in reducing seizure activity^{156,178,188}.

Hearing and vision

Conductive hearing loss is common in individuals with DS, with a high prevalence of otitis media with effusion^{189,190}. Baseline hearing tests should be performed postnatally using, for example, brainstem auditory acoustic response, and then every 6 months until school age and at least annually thereafter¹⁵⁷. Early recognition and treatment can reduce the risk of later hearing loss¹⁹¹. Sensorineural loss becomes increasingly common in adulthood. Treatment with hearing aids and cochlear implantation has been successful. The use of speech therapy, communication aids and sign language, including the language programme Makaton, can also be beneficial¹⁶⁰.

Because there is increased risk of refractive errors, cataracts (congenital and developmental), keratoconus and amblyopia in individuals with DS, ophthalmological examination should be performed at birth and regularly throughout life, ideally every 1–2 years¹⁵⁷. Treatment and corrective aids should be given promptly¹⁵⁷. Cataract surgery is routinely performed, usually with good outcomes^{192,193}.

Atlantoaxial instability

Atlantoaxial subluxation (misalignment of the first and second cervical vertebrae) develops in ~1–2% of children with DS¹⁹⁴. If the condition is present, parents should be advised that participation in sports increases the risk of spinal cord injury in the child. Symptoms such as neck pain, weakness, spasticity, gait difficulties and hyperreflexia should be evaluated with cervical spine radiography¹⁵⁷.

Mental health

Individuals with DS have increased prevalence of behavioural and mental health problems compared with the general population¹⁹⁵, in particular, depressive and anxiety disorders^{196,197}. A small proportion of adolescents and young adults with DS undergo acute regression (also known as Down syndrome disintegrative disorder), which involves a loss of skills and independence compared with their previous levels of functioning. At present, the cause of this decline is unknown, although it often seems to occur after exposure to emotional stressors¹⁹⁸. To date, there is no definitive treatment for this presentation.

Diagnosis of mental health issues in individuals with DS can be complicated by the presence of intellectual disability, communication difficulties and atypical symptoms¹⁹⁹. The effect of psychological stressors should not be underestimated, including transitions in accommodation, school or care arrangements, and bereavement^{166,200}. Treatment for mental illness should be based on standard clinical guidelines, and individuals with DS show positive responses to behavioural interventions, psychotropic medication and psychological therapy.

Neurodevelopment

DS is the most common genetic cause of intellectual disability, with the majority of individuals with DS classified as having mild–moderate disability. Their cognitive profile demonstrates strengths in visual learning, but weaknesses in expressive language, verbal working memory, and episodic memory²⁰¹. However, there is a wide range in cognitive function, with variations in IQ, language, attention, memory and functional abilities.

Individuals with DS often have autism spectrum disorder (~10–15%) and attention-deficit–hyperactivity disorder (ADHD; ~6%) and appropriate screens for these should be performed^{160,202}. Clinical presentations may differ from those of the general population and assessments may require input from specialists. Standard treatments for ADHD are recommended, although there can be a less marked response to stimulant medication in individuals with intellectual disability than in those without intellectual disability²⁰³.

Prenatal therapy to improve neurocognition.—Increasing attention has been paid to using prenatal diagnosis of DS as an opportunity to provide antenatal therapy to improve neurocognition^{204,205}. Impairment of neurogenesis in individuals with DS begins prenatally^{206–208} and is one of several factors²⁰⁹ that result in abnormalities in fetal brain growth and shape that are recognizable in third trimester prenatal ultrasound and MR images²¹⁰. Thus, treatment initiated in fetal or early neonatal life is hypothesized to have maximal effect²¹¹ (the ethical issues posed by treatment of fetuses with DS are discussed elsewhere²¹²).

To date, most experiments involving fetal therapy have been performed using mouse models of DS. Antenatal treatments that have resulted in postnatal improvement in learning and neurobehaviour in Ts65Dn mice include dosing with the vasoactive intestinal pep-tides NAPVSIPQ (NAP) and SALLRSIPA (SAL)²¹³, maternal dietary choline supplementation²¹⁴, and inhibition of DYRK1A. For example, prenatal treatment with the

DYRK1A inhibitor EGCG, which crosses both the placental and blood–brain barriers in mice, improved some aspects of the cranial vault morphological defects in Ts65Dn mice²¹⁵. Furthermore, oral administration of the novel DYRK1A inhibitor ALGERNON (altered generation of neurons) to pregnant Ts1Cje dams from embryonic day 10 (E10) to E15 improved neurogenesis, corticogenesis and behavioural performance of their pups, possibly by enhancing the proliferation of neural stem cells²¹⁶.

Other potential therapeutic targets have been identified from studies of mouse models. For example, mitochondrial dysfunction in fibroblasts from human fetuses with DS is reversed by activation of the PGC1α pathway using metformin²¹⁷. The mRNA and protein levels of PGC1α are markedly reduced in fibroblasts from fetuses with DS, and metformin treatment increased oxygen consumption and cellular ATP concentration, improved respiratory activity, increased mitochondrial membrane potential and reversed mitochondrial ultrastructural abnormalities in these cells²¹⁷. Metformin already has FDA regulatory approval for the treatment of maternal gestational diabetes, so its use in clinical trials in pregnant women carrying fetuses with DS would potentially face fewer regulatory hurdles. Studies in mouse models of DS have also shown that prenatal treatment with the selective serotonin reuptake inhibitor fluoxetine restores hippocampal neurogenesis and connectivity, granule cell number and dendritic patterns, and hippocampus-dependent memory functions²¹⁸. A clinical trial of high-dose fluoxetine in pregnant women (without psychiatric disorders) is ongoing at the University of Texas (USA)²¹¹, although no information about the trial is publicly available. However, fluoxetine use in the first trimester may have adverse effects, such as increasing the risk of congenital heart disease and other malformations²¹⁹.

Apigenin, a natural flavone found in citrus fruits and leafy green vegetables, also shows promise in reducing oxidative stress and improving total oxidative capacity in human cells from fetuses with trisomy 21, as well as improving some behaviours in neonatal and adult Ts1Cje mice treated prenatally²⁰⁴. The recognition of the possibilities for improving fetal brain development, and testing of an increasing number of compounds for eventual use in clinical trials, make this a very exciting time in DS research, although recommendations for supplementation cannot be made owing to a paucity of evidence.

Immune and haematological systems

Individuals with DS are more prone to infections, especially of the respiratory tract^{220–222}. Infections should be recognized and treated promptly¹⁵⁷. There is no contraindication to immunizations, and the standard childhood schedule should be followed.

Individuals with DS also show a predisposition to autoimmune diseases, particularly coeliac disease^{160,223}. Testing the levels of immunoglobulin A antibodies to tissue transglutaminase 2 is recommended if symptoms of coeliac disease are present¹⁵⁷, but screening of asymptomatic individuals has not proved cost-effective²²⁴. Type 1 diabetes mellitus, alopecia areata and Addison disease have also been reported in individuals with DS at higher rates than in the general population.

Children with DS are at a markedly increased risk of developing acute leukaemia, compared with children without DS. Transient myeloproliferative disorder (TMD), also referred to as transient leukaemia of DS, is observed in 5–30% of individuals with DS before 3 months of age, and a blood count and film should be performed within 3 days of birth to enable the identification and monitoring of this disorder²²⁵. Although TMD usually resolves without treatment in the first few months of life, it seems to increase the risk of developing leukaemia by 5 years of age, and it is estimated that 20–30% of individuals with TMD will go on to develop myeloid leukaemia of DS^{157,226,227}.

Quality of life

Research into the quality of life of individuals with DS is limited, although it is recognized that stigma and cultural norms can be barriers to the active participation of individuals with DS in the community. If adequately supported during their life, many individuals with DS can live fairly independently. The perspectives of family members of individuals with DS have been somewhat studied²²⁸. Support groups and DS networks can provide valuable input, and individuals with DS and their families should be aware of local and national organizations.

Children with DS are now regularly educated within mainstream schools, often with additional one-to-one or specific Special Educational Needs support²²⁹. Some children with DS can gain additional benefits in terms of language and literacy skills when educated in inclusion classrooms rather than in substantially separate classrooms, whereas others may require a specialist school²³⁰.

Many individuals with DS are employed and report fairly high levels of satisfaction, and many have become successful professionals, including artists, speakers and actors. However, there is a disparity between the number of individuals with DS who wish to work and those who have secured employment. They may access voluntary work and this seems to be more common than in the general adult population. Cultural assumptions, social care cuts and inadequate adjustments in work can make it difficult for individuals with DS to access employment^{231,232}.

Quality of life can be affected by physical health, and proactive screening programmes and early intervention can reduce the negative effect of prolonged hospitalizations and ill health. Recognition and correction of hearing and vision problems can reduce the negative effect on communication and development²³³. Obesity, cervical spine abnormalities and muscular difficulties may contribute to the exclusion of individuals with DS from physical activity, and in the USA, children with DS have lower levels of physical activity than their peers²³⁴. However, the majority would be able to participate in exercise programmes if available, which can have a positive effect on health and wellbeing²³⁵.

Owing to early cognitive decline, many adults with DS have increased support needs as they age. Adequate proactive support, with regular reassessment of needs and services set up to anticipate functional impairments, is crucial for their quality of life.

Outlook

Although the lives of individuals with DS have been much improved by the evolution of medical care and the benefits of societal changes, there is no treatment for DS at present that is based on the current understanding of the molecular pathophysiology of DS. Therefore, to design effective therapies, further research is needed to improve understanding of the biology of each symptom and feature of the syndrome. The most important topics for further research are discussed below.

Understanding the contribution of genetic variation to the different and variable phenotypic characteristics and manifestations of DS is an important goal that could be achieved by genome sequencing of thousands of individuals with DS, and linking the genetic variation with the detailed phenotypic characterization of each individual⁶. This effort is based on the reasonable hypothesis that genomic differences are major determinants of the extensive phenotypic variation. Furthermore, genes and other functional genomic elements that in three copies contribute to the phenotype of DS need to be identified. There is supportive evidence that genes causing disease states with haploinsufficiency, in which one functional copy is not sufficient, may also be important when present in three copies, as is the case with DS. For example, *DYRK1A* is haploinsufficient (probability of loss-of-function intolerant (pLI) score of 1), and the loss of one copy of *DYRK1A* causes intellectual disability²³⁶. Several studies have also shown that an extra copy of *DYRK1A* is involved in the biological dysfunction that results from trisomy 21 (REF²³⁷). However, the pLI score may not be an absolute discriminator of genes involved in the pathophysiology of DS. For example, *APP* has been strongly linked to AD in individuals with DS¹²⁰ but has a pLI score of 0.06, which is compatible with a haplosufficient gene. Furthermore, *HMGNI*, a gene that is linked to the transcriptional dysregulation in trisomy 21(REF²³⁸), has a pLI score of 0.22. In addition to the causative contribution of specific genes on HSA21, the phenotypic contribution of the extra-chromosomal material, regardless of its gene content, needs to be investigated.

Experimental models, such as the mouse and other organisms⁷, need to be used to study the brain and other organs in the different stages of development. Advances in single-cell technologies²³⁹ that enable study of the genome, transcriptome, histone modifications, chromatin contacts and protein levels in individual cells will certainly provide insights into the cellular mechanisms of the altered developmental pathways in DS and the effect of chromatin conformation on the dysregulation of gene expression.

Experimental efforts to modify the dysregulated expression of genes on HSA21 and other chromosomes may result in novel therapeutic approaches that should be explored further. For example, experimental silencing of large chromosomal regions by introducing the X chromosome-inactivation effector *XIST* on one copy of HSA21, or silencing one of the three alleles of *HMGNI*, *DYRK1A* or *APP*, may rescue some phenotypic characteristics of DS.

Early-onset AD in individuals with DS is genetically analogous to some forms of autosomal dominant AD, and the amyloid pathway is strongly implicated in AD pathology due to *APP* triplication. Therefore, amyloid-targeting drugs should be trialled in individuals with DS, as they are at very high risk of early-onset AD, preferably before any symptoms develop

in order to prevent or delay the onset of dementia¹¹⁵. PET imaging of amyloid or tau deposits in the brain^{240,241} or measures of Aβ peptide ratios in cerebrospinal fluid²⁴² have been shown to be feasible biomarkers for patient stratification, whereas plasma levels of the neurodegeneration markers neurofilament light chain and neuronal pentraxin 2 show promise as biomarkers of treatment response^{243–245}.

Population-based, longitudinal, large-scale cohort studies following individuals with DS would be helpful to the further understanding of the co-occurring medical conditions, family attitudes, educational achievement, individual experiences, and societal successes that now exist for individuals with DS. Increasingly sophisticated analytical methods combined with improved genetic models offer hope for more translation of promising therapies.

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Competing interests

S.E.A. is the co-founder and CEO of MediGenome, a clinical and laboratory diagnostic company. B.G.S. occasionally consults on the topic of Down syndrome through Gerson Lehrman Group. B.G.S. receives remuneration from Down syndrome non-profit organizations for speaking engagements and associated travel expenses. B.G.S. receives annual royalties from Woodbine House, Inc., for the publication of his book, *Fasten Your Seatbelt: A Crash Course on Down Syndrome for Brothers and Sisters*. Within the past 2 years, B.G.S. has received research funding from F. Hoffmann-La Roche, Inc., and LuMind IDSC Down Syndrome Foundation to conduct clinical trials for people with Down syndrome. B.G.S. is occasionally asked to serve as an expert witness in legal cases where Down syndrome is discussed. B.G.S. serves in a non-paid capacity on the Honorary Board of Directors for the Massachusetts Down Syndrome Congress and the Professional Advisory Committee for the National Center for Prenatal and Postnatal Down Syndrome Resources. B.G.S. has a sister with Down syndrome. M.S.R. is a consultant to AC Immune SA. A.S. has consulted for Roche Pharmaceuticals, ONO Pharma, Aelis Farma and AC Immune, and he serves on the Scientific Advisory Board of ProMIS Neurosciences. A.S. and S.E.P. provide clinical services within the UK National Health Service to individuals with Down syndrome. The remaining authors declare no competing interests.

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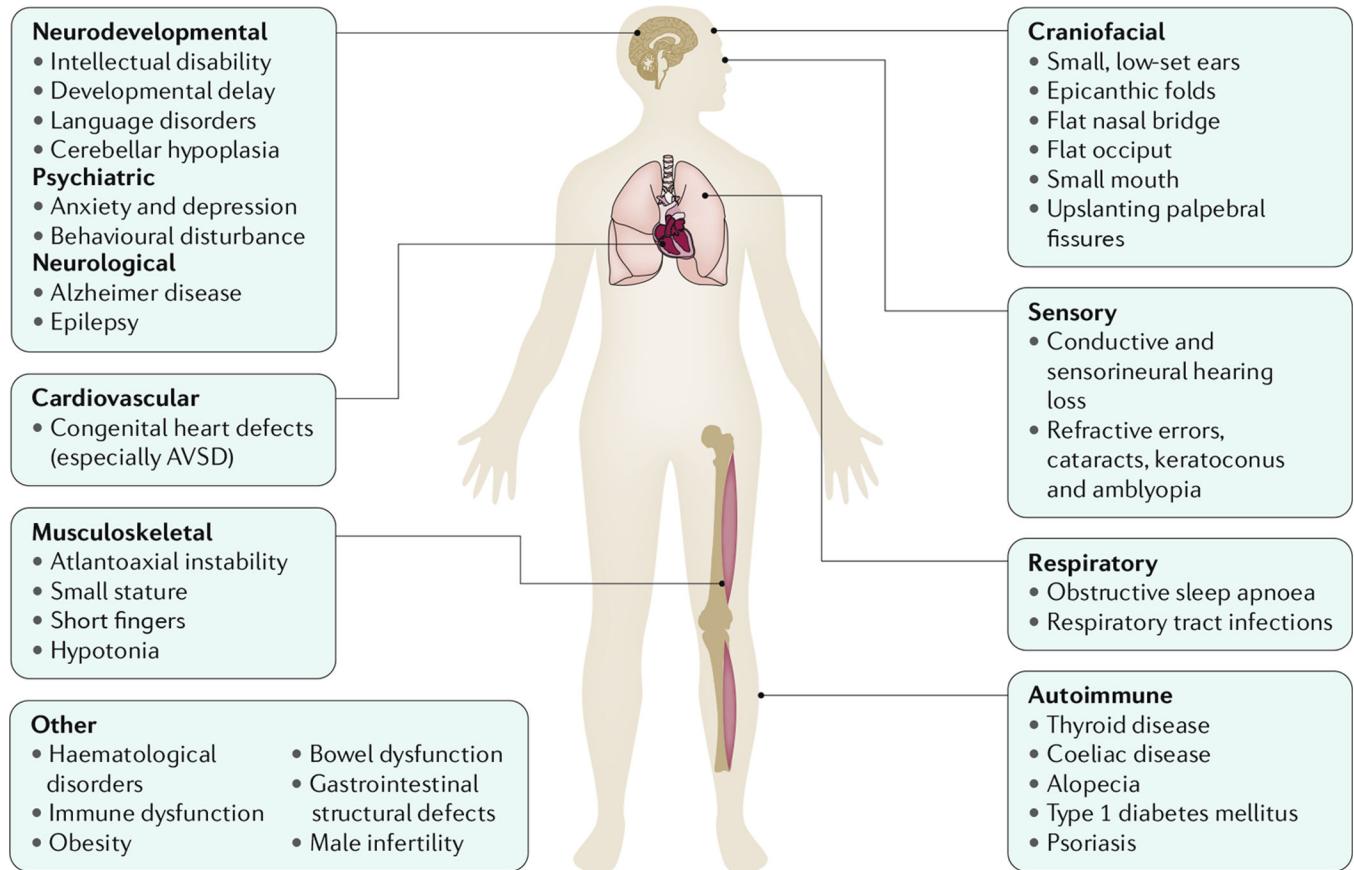


Fig. 1 | Symptoms and manifestations in Down syndrome.

Individuals with trisomy 21 (the presence of a supernumerary chromosome 21; also known as Down syndrome (DS)) present with a distinct collection of symptoms and manifestations that affect multiple body systems, although variation exists between individuals. Individuals with DS are generally of short stature, with short fingers, hypotonia and atlantoaxial instability. Facial characteristics include the presence of epicanthic folds, flat nasal bridge and occiput, small mouth and ears, and up-slanting palpebral fissures. Congenital heart defects are common, particularly atrioventricular septal defect (AVSD). Individuals with DS are also more likely to develop certain health conditions compared with the general population, including hypothyroidism, obstructive sleep apnoea, epilepsy, hearing and vision problems, haematological disorders (including leukaemia), recurrent infections, anxiety disorders, and early-onset Alzheimer disease.

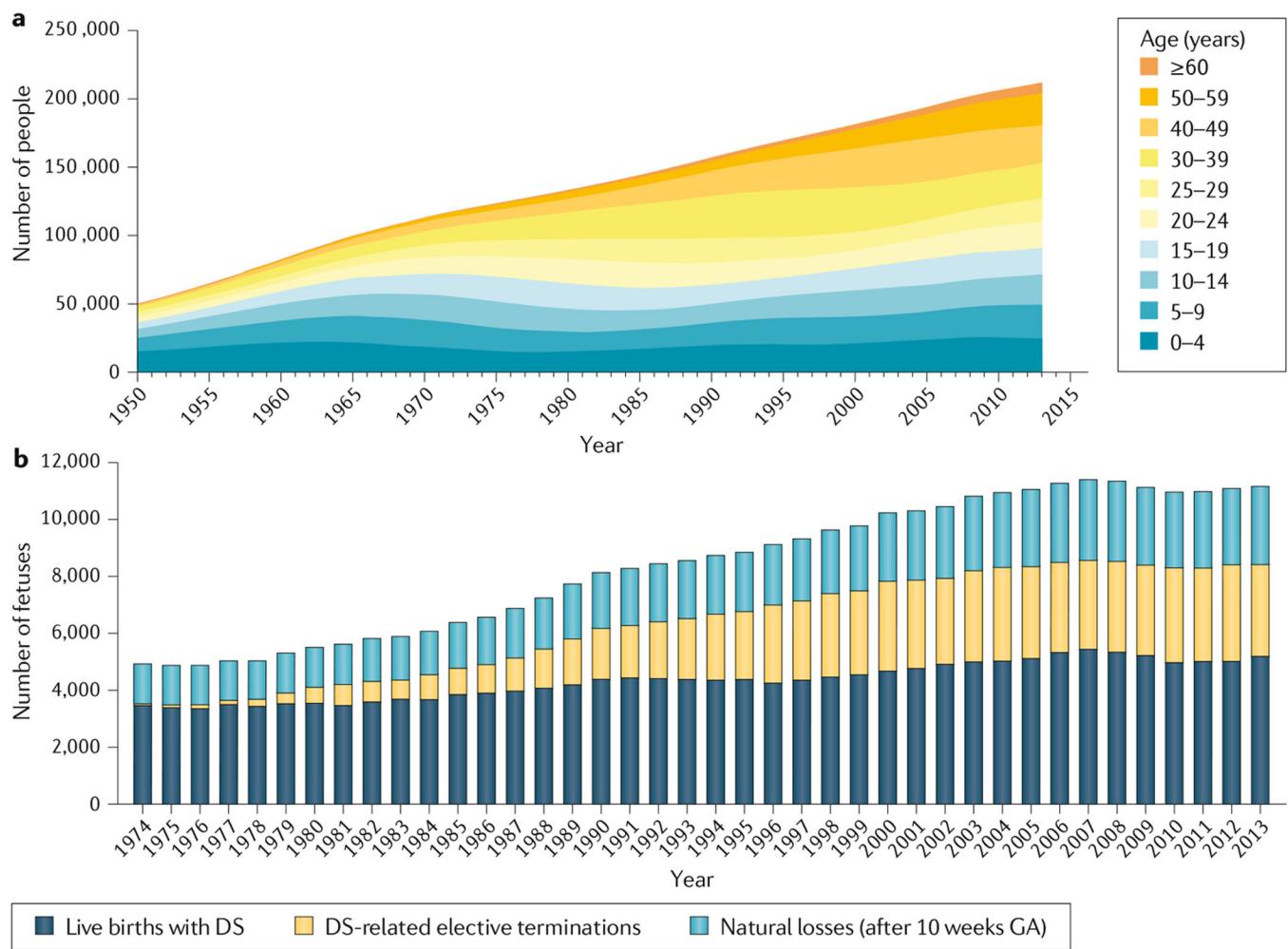


Fig. 2 |. Prevalence of DS and pregnancy outcomes in the USA.

a | Prevalence of Down syndrome (DS) in the USA for the period 1950–2013. This graph combines the prevalence data for 1950–2010 in the USA⁹ with unpublished prevalence data for 2011–2013 for the same region¹⁰. **b |** Pregnancy outcomes in the USA for the period 1974–2013. This graph combines estimates of live births, natural losses and elective terminations in women carrying a fetus with DS after 10 weeks gestation for the period 1974–2010 in the USA¹⁷ with unpublished data for 2011–2013 (REF.¹⁰). GA, gestational age. Part a adapted from REF.¹¹, Springer Nature Limited. Part b adapted with permission from REF.¹⁷, Wiley-VCH.

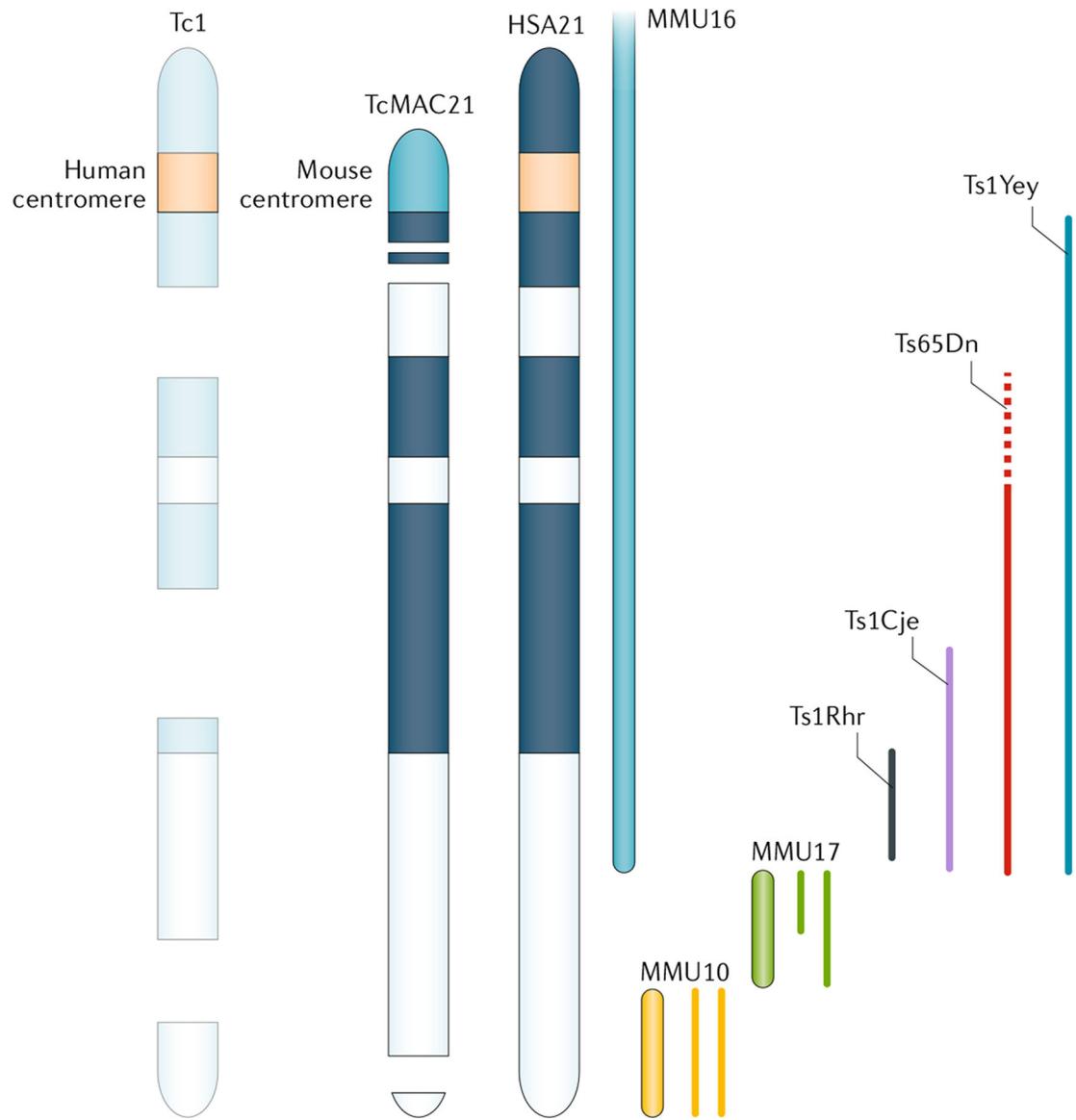


Fig. 3 |. Conserved synteny of human chromosome 21 with mouse chromosomes and mouse models of trisomy 21.

Down syndrome (DS) results from the presence of a supernumerary *Homo sapiens* chromosome 21 (HSA21). More than 20 mouse models of DS have been created, which are designed to overexpress part of or a complete HSA21, or the orthologous mouse genomic regions^{7,91,246}. Mouse orthologues of HSA21 genes occur on *Mus musculus* chromosome 16 (MMU16), MMU17 and MMU10. Tc1 mice carry a mutated HSA21 and are mosaic animals, with a mix of trisomic and euploid cells that is unique to each individual, apparently due to suboptimal function of the human centromere in mice. Ts65Dn animals contain a duplication of ~140 genes on MMU16, some of which are not orthologous to HSA21^{247,248} (dashed line). TcMAC21 animals contain the long arm of HSA21 (HSA21q) as a mouse artificial chromosome (that is, with a mouse centromere to ensure that the chromosome is retained in every cell); however, this artificial chromosome contains deletions that affect ~8% of HSA21q genes. All of these models except Ts65Dn

are direct duplications; that is, the genes in each are trisomic but they do not contain an extra chromosome or centromere. Ts1Rhr, Ts1Cje, Ts1Yey and Ts65Dn mouse models are discussed in the text.

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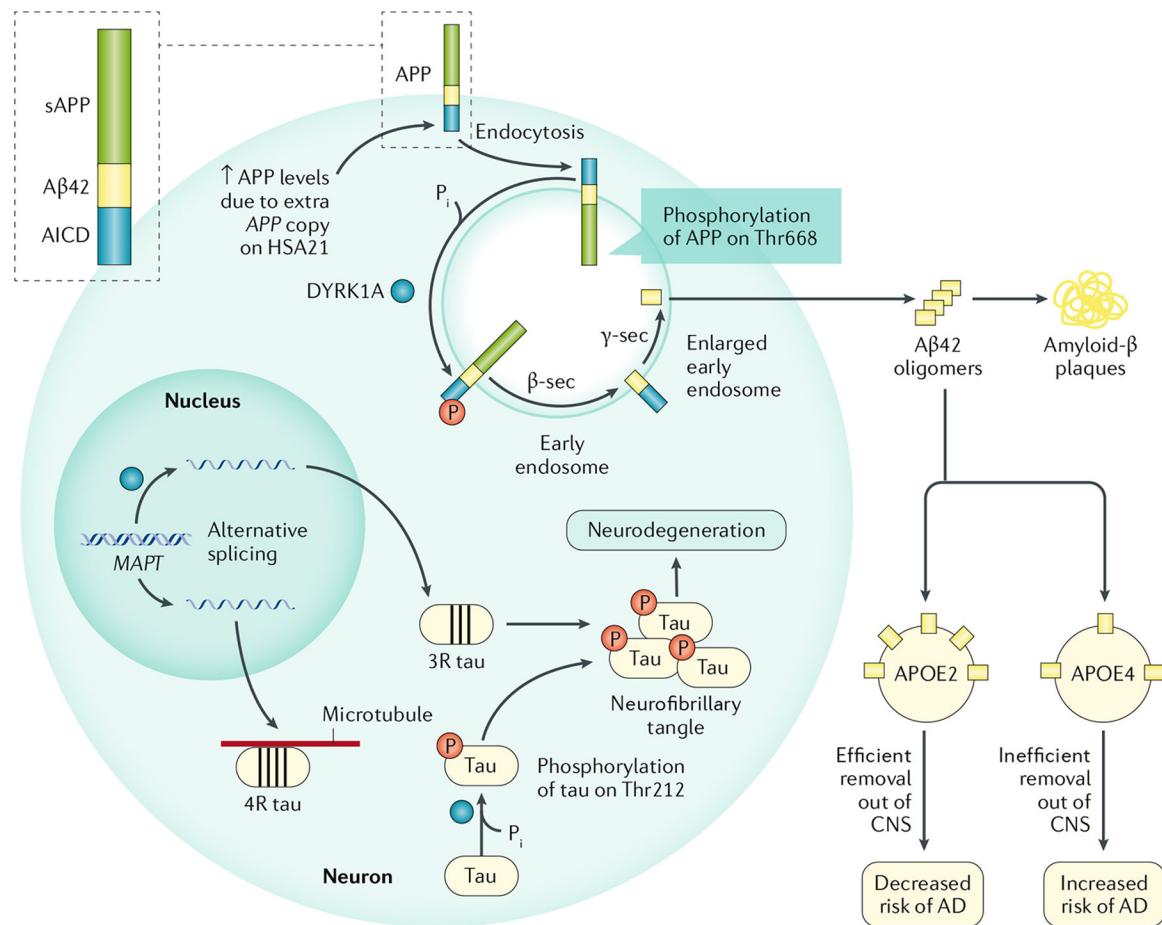


Fig. 4 | Mechanisms of Alzheimer disease in Down syndrome.

The increased gene dosage of *APP* (encoding amyloid precursor protein (APP)) and *DYRK1A* (encoding dual-specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A)) on *Homo sapiens* chromosome 21 (HSA21) in individuals with Down syndrome (DS; trisomy 21) increases their risk of developing Alzheimer disease (AD). APP can undergo non-amyloidogenic processing (not shown) and amyloidogenic processing by β -secretase 1 (β -sec) and γ -secretase (γ -sec) to produce the neurotoxic A β 42 peptide. The increased APP levels result in higher levels of A β 42, and phosphorylation of APP at Thr668 by DYRK1A increases the amyloidogenic processing of APP. DYRK1A has other targets in the cell, including splicing factors and the microtubule-binding protein tau. DYRK1A phosphorylation of splicing factors alters the splicing of the *MAPT*mRNA (encoding tau), resulting in increased levels of tau containing three microtubule-binding domains (three-repeat (3R) tau) and reduced levels of 4R tau. This imbalance leads to neurofibrillary tangle (NFT) formation, possibly due to the lower binding affinity of 3R tau for microtubules. Furthermore, DYRK1A phosphorylation of Thr212 in tau alters the conformation of the protein, reducing the affinity of tau for microtubules and leading to NFT formation. The presence of the e4 allele of *APOE* (*APOE**e4) alters the processing, deposition and clearance of A β ²⁴⁹, and is therefore a major risk allele for AD. AICD, APP intracellular domain; APOE, apolipoprotein E; CNS, central nervous system; P_i, inorganic phosphate; sAPP, soluble amyloid precursor protein.

