

Down syndrome—recent progress and future prospects

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Received December 19, 2008; Revised December 19, 2008; Accepted January 5, 2009

Down syndrome (DS) is caused by trisomy of chromosome 21 (Hsa21) and is associated with a number of deleterious phenotypes, including learning disability, heart defects, early-onset Alzheimer's disease and childhood leukaemia. Individuals with DS are affected by these phenotypes to a variable extent; understanding the cause of this variation is a key challenge. Here, we review recent research progress in DS, both in patients and relevant animal models. In particular, we highlight exciting advances in therapy to improve cognitive function in people with DS and the significant developments in understanding the gene content of Hsa21. Moreover, we discuss future research directions in light of new technologies. In particular, the use of chromosome engineering to generate new trisomic mouse models and large-scale studies of genotype–phenotype relationships in patients are likely to significantly contribute to the future understanding of DS.

INTRODUCTION

Down syndrome (DS) is caused by trisomy of human chromosome 21 (Hsa21). Approximately 0.45% of human conceptions are trisomic for Hsa21 (1). The incidence of trisomy is influenced by maternal age and differs between populations (between 1 in 319 and 1 in 1000 live births are trisomic for Hsa21) (2–6). Trisomic fetuses are at an elevated risk of miscarriage, and people with DS have an increased risk of developing several medical conditions (7). Recent advances in medical treatment and social inclusion have significantly increased the life expectancy of people with DS. In economically developed countries, the average life span of people who are trisomic for Hsa21 is now greater than 55 years (8). In this review, we will discuss novel findings in the understanding of DS and highlight future important avenues of research.

The additional copy of Hsa21, in people with DS, is proposed to result in the increased expression of many of the genes encoded on this chromosome. The imbalance in expression of Hsa21 and non-Hsa21 genes is hypothesized to result in the many phenotypes that characterize DS. However, only some of the Hsa21 genes are likely to be dosage-sensitive, such that the phenotype they confer is

altered by gene-copy number. Thus to understand DS, it is crucial both to understand the genomic content of Hsa21 and to evaluate how the expression levels of these genes are altered by the presence of a third copy of Hsa21. There have been a number of recent advances in genomics relevant to DS. For example, the traditional definition of a gene has been modified (Box 1). A number of fusion transcripts that are encoded by two or more genes previously considered to be separate have been reported, such as the transcript encoded by exons from the Hsa21, *DONSON* and *ATP50* genes (9). Whether these transcripts represent novel genes has yet to be determined. However, the number of genes recognized on Hsa21 is likely to continue to increase from the current count of more than 400 (10). In particular, as algorithms to identify non-coding RNAs (e.g. microRNAs) improve, the number of recognized genes may increase. Five microRNAs have been identified on Hsa21 (11,12). MicroRNAs regulate the expression of other genes (13), and their role in DS is not fully understood. Spatial and temporal mapping of the Hsa21 gene expression is also critical to the understanding of DS. The increase in expression of some Hsa21 genes caused by trisomy of Hsa21 has been recently shown to lie within the range of natural variations in the expression of these genes in the euploid population

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(14,15). Similar findings have also been reported in the Ts(17¹⁶)65Dn (Ts65Dn) mouse model of DS (Fig. 1) (16).

Box 1: What is a gene?

The definition of a gene has shifted over the past 100 years since it was first coined by Wilhelm Johannsen in 1909, based on the ideas of Mendel, de Vries, Correns and Tschermak. Their original theoretical definition of the gene being 'the smallest unit of genetic inheritance' remains the cornerstone of our understanding; however, the definition has grown with our knowledge of molecular biology. The gene has recently been defined as 'a union of genomic sequences encoding a coherent set of potentially overlapping functional products' (133). Splicing generates multiple transcripts from one gene. Moreover, exons from genes previously considered to be separate may be spliced together to generate novel transcripts (9). How to classify these fusion transcripts is a significant challenge. In addition, alternative transcription start sites that generate novel 5' untranslated regions continue to be discovered, even for well-characterized genes (134). Although many of these novel transcripts are rare and their functional importance is not understood, our definition of a gene must encompass the observed diversity of the genome.

This suggests that these genes are unlikely to be candidates for the dosage-sensitive genes underlying DS phenotypes in the tissues investigated.

Trisomy of Hsa21 is associated with a small number of conserved features, occurring in all individuals, including mild-to-moderate learning disability, craniofacial abnormalities and hypotonia in early infancy (17). Although these phenotypes are always found in people with DS, the degree to which an individual is affected varies. Additionally, trisomy of Hsa21 is also associated with variant phenotypes that only affect some people with DS, including atrioventricular septal defects (AVSDs) in the heart, acute megakaryoblastic leukaemia (AMKL) and a decrease in the incidence of some solid tumours. This phenotypic variation is likely to be caused by a combination of environmental and genetic causes. Genetic polymorphisms in both Hsa21 and non-Hsa21 genes may account for much of this variation. Genome-wide association studies to identify these polymorphisms constitute a promising strategy to gain novel insights into the pathology of DS.

A central goal of DS research is to understand which of the genes on Hsa21, when present in three copies, lead to each of the different DS-associated phenotypes, and to elucidate how increased expression leads to the molecular, cellular and physiological changes underlying DS pathology. Two distinct approaches are being taken to address these issues. First, genomic association studies, such as that recently published by Lyle *et al* (18), may point to genes that play an important role in pathology. Secondly, a number of animal models of Hsa21 trisomy have been generated. Recent advances in chromosome engineering have led to the establishment of mice trisomic for different sets of mouse genes syntenic to Hsa21, and a mouse strain, Tc(Hsa21)1TybEmcf (Tc1), carrying most of Hsa21, as a freely segregating chromosome

(Fig. 1) (19–27). These strains are being used both to map dosage-sensitive genes on Hsa21 and to understand pathological mechanisms. Here, we review recent advances in the understanding of DS-associated phenotypes and the development of therapeutic strategies to treat them.

RECENT ADVANCES IN UNDERSTANDING PHENOTYPES ASSOCIATED WITH DS

Development

Trisomy of Hsa21 has a significant impact on the development of many tissues, most notably the heart and the brain. A recent paper has suggested that trisomy of the Hsa21 genes, dual-specificity tyrosine-(Y)-phosphorylation-regulated kinase 1A (*DYRK1A*) and regulator of calcineurin 1 (*RCAN1*), may have an impact on the development of multiple tissues (28). *DYRK1A* is a priming kinase that facilitates the further phosphorylation of numerous proteins by other kinases (Fig. 2) (29–38). It is up-regulated in a number of tissues from people with DS (39,40). *RCAN1* is a regulator of the protein phosphatase calcineurin (41). Crabtree and colleagues hypothesized that trisomy of these two genes may act synergistically to alter signalling via the NFAT family of transcription factors (28). In an independent study, increased *DYRK1A* gene dosage was shown to decrease the expression level of RE1-silencing transcription factor (*REST*) (42). As *REST* is required both to maintain pluripotency and to facilitate neuronal differentiation, a perturbation in *REST* expression may alter the development of many cell types. Indeed, over-expression of *DYRK1A* in some animal models is associated with a number of phenotypes, including heart defects and abnormal learning and memory (28,33,43–45). However, not all animal models that over-express *DYRK1A* exhibit these defects, suggesting that polymorphisms or differences in the expression of other genes influence the outcome of *DYRK1A* trisomy (24).

Trisomy of Hsa21 is associated with a reduction in brain volume, the size of the hippocampus and cerebellum being particularly affected (46–49). A similar phenotype is also observed in the Ts65Dn model (50). Recent studies have started to elucidate the developmental mechanisms underlying these important phenotypes. Trisomic granule cell precursors from the cerebellum have a reduced mitogenic response to the morphogen sonic hedgehog (51). This was shown to underlie the reduced number of cerebellar granular cells observed in the Ts65Dn mouse model of DS. Hypocellularity in the hippocampus also has a developmental origin (52,53). Abnormalities in cell-cycle length, apoptosis and neocortical neurogenesis have been shown to contribute to this phenotype (53–55). The reduced level of neurogenesis in Ts65Dn adult hippocampus can be ameliorated by treatment with the antidepressant fluoxetine, which is a serotonin reuptake inhibitor (56). Fluoxetine may promote neurogenesis via a number of potential mechanisms, including a direct effect on serotonin levels or via an indirect effect on behaviour. Whether this drug has similar effect during embryonic development has yet to be determined.

Ts65Dn pups exhibit a delay in attaining several developmental milestones, such as forelimb grip and the righting reflex, mimicking the developmental delay observed in

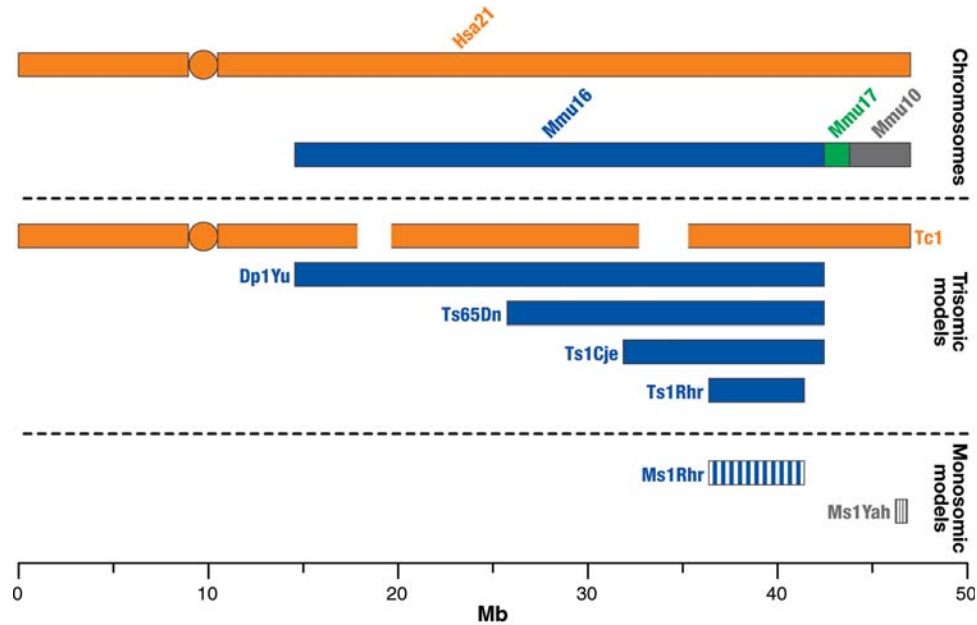


Figure 1. Mouse models of Hsa21 trisomy and monosomy. Hsa21 (orange) is syntenic with regions of mouse chromosomes 16 (Mmu16, blue), 17 (Mmu 17, green) and 10 (Mmu10, grey). The Tc1 mouse model carries a freely segregating copy of Hsa21, which has two deleted regions, such that the model is trisomic for the majority of genes on Hsa21. The Dp1Yu, Ts65Dn, Ts1Cje and Ts1Rhr mouse models contain an additional copy of regions of mouse chromosome 16 that are syntenic with Hsa21, such that they are trisomic for a proportion of Hsa21 genes. The Ms1Rhr mouse model contains a deletion of a region of Mmu16; the Ms1Yah mouse model contains a deletion of a region of Mmu10. Hence, these models are monosomic for the genes in these deleted Hsa21 syntenic segments.

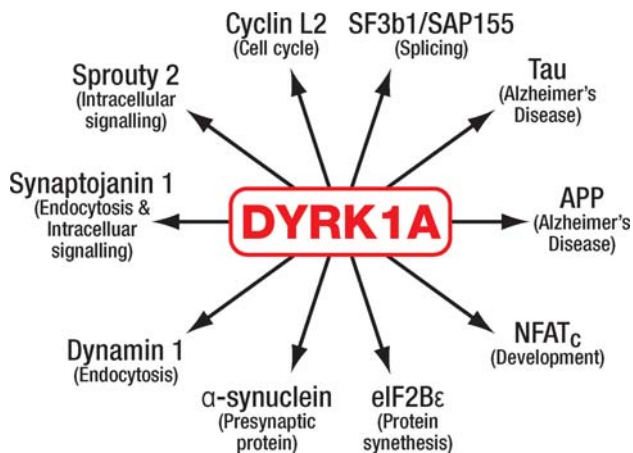


Figure 2. Phosphorylation targets of DYRK1A. The Hsa21-encoded kinase DYRK1A has been shown to phosphorylate a multitude of targets, which have been implicated in a number of biological processes and DS-associated phenotypes, including endocytosis and AD.

babies with DS (57). A recent report has demonstrated that treatment of Ts65Dn embryos with two neuroprotective peptides reduced the delay in achieving a number of sensory and motor developmental milestones during early post-natal development (58).

People with DS exhibit craniofacial dysmorphism, including a mandible of reduced size. This phenotype is also observed in the Ts65Dn and Tc1 models (26,59). In the Ts65Dn model, craniofacial dysmorphism is present from early post-natal development and may be related to specific changes in bone development (60,61). The small mandible

in people with DS may be caused by migration and proliferation defects in mandible precursor (neural crest) cells in the developing embryo, related to an altered response to sonic hedgehog (62).

Learning and memory

All people with DS have a mild-to-moderate learning disability. Over-expression of a number of Hsa21 genes, including DYRK1A, synaptojanin 1 and single-minded homologue 2 (SIM2), results in learning and memory defects in mouse models, suggesting that trisomy of these genes may contribute to learning disability in people with DS (43,45,63,64). In addition, trisomy of neuronal channel proteins, such as G-protein-coupled inward-rectifying potassium channel subunit 2 (*GIRK2*), may also influence learning in people with DS (65–67). Recent work has demonstrated that trisomy of a segment of mouse chromosome 16 (*Mmu16*) containing 33 genes including *DYRK1A*, *GIRK2* and *SIM2* was necessary, but not sufficient for the hippocampal-based learning deficits in the Ts65Dn mouse model (68). These data indicate that trisomy of multiple Hsa21 genes is required for the deficits in learning associated with DS. Moreover, Hsa21 trisomy may independently impact on multiple learning pathways.

Recent work on the Tc1 transchromosomal mouse model of DS has examined in detail the learning pathways affected by trisomy of Hsa21 (26,69). The Tc1 transchromosomal model exhibits abnormalities in short-term but not in long-term hippocampal-dependent learning. The learning deficits are correlated with specific abnormalities in long-term potentiation (LTP) in the dentate gyrus of the hippocampus. LTP is an electrophysiological process proposed to be the cellular

basis of learning and memory (70). These data provide insight into which learning mechanisms may be affected by Hsa21 trisomy and can be used to further understand their genetic cause. Structural abnormalities may contribute to these deficits in learning and memory. Indeed, a correlation between specific synaptic abnormalities in the hippocampus of the Ts(16C-tel)1Cje (Ts1Cje) mouse and a defect in LTP has been reported (71). Moreover, a recent paper has demonstrated an alteration in the amounts of a number of synaptic components in the hippocampus of the Ts65Dn mouse (72).

Alzheimer's disease

People with DS have a greatly increased risk of early-onset Alzheimer's disease (AD). By the age of 60, between 50 and 70% of the people with DS develop dementia (73–77). The known AD risk factor amyloid precursor protein (*APP*) is encoded on Hsa21. Trisomy of *APP* is likely to make a significant contribution to the increased frequency of dementia in people with DS. Indeed, triplication of a short segment of Hsa21 that includes *APP* in people without DS has been recently shown to be associated with early-onset AD. A number of features of neurodegeneration have been observed in mouse models of DS (78–86). Loss of basal forebrain cholinergic neurons (BFCNs) occurs early in AD and also is observed in the Ts65Dn mouse model (87). Degeneration of BFCNs in Ts65Dn mice is dependent on trisomy of *APP* and is mediated by the effect of increased *APP* expression of retrograde axonal transport (83).

Hsa21 genes other than *APP* may also contribute to the early onset of AD in people with DS (33,34,40,88–97). Indeed, the Ts1Cje mouse model, which is not trisomic for *APP*, exhibits tau hyperphosphorylation, an early sign of AD (98). Recent evidence suggests that trisomy of *DYRK1A* may contribute to the development of AD in people with DS. *DYRK1A* can phosphorylate Tau at a key priming site that permits its hyperphosphorylation (33,36,40,95). *DYRK1A* may also influence the alternative splicing of Tau and the phosphorylation of *APP* (34,99). A reduction in the level of protein phosphatase 2A and a decrease in the activity of α -secretase in the brains of people with DS have also been reported, both of which may contribute to AD in this population (94,100). Further studies are required to determine the identity of the trisomic genes that contribute to these phenotypes.

Heart defects

Trisomy of Hsa21 is associated with a number of congenital heart defects, the most common being AVSD that occurs in ~20% of the people with DS (101). Mutations in the non-Hsa21 *CRELD1* gene may contribute to the development of AVSD in DS (102). *CRELD1* has also been linked to AVSDs by mapping the deletion breakpoints, on chromosome 3, in people with 3p-syndrome. Further studies are required to determine the identity of other genes that are important for heart development in people with DS. A number of Hsa21 trisomy mouse models exhibit heart defects similar to those observed in DS, suggesting that trisomy of one or more of the approximately 100 genes common to these models influences development of the heart (22,26,103,104).

Leukaemia and cancer

DS increases the risk of developing AMKL and acute lymphoblastic leukaemia (ALL). Approximately 10% of the DS newborns present with a transient myeloproliferative disorder (TMD), characterized by a clonal population of megakaryoblasts in the blood. This transient disease usually spontaneously resolves; however, 10–20% of the DS patients with TMD develop AMKL before 4 years of age (reviewed in 105). The development of TMD requires both trisomy 21 and mutations in the transcription factor GATA1 (106,107). It is likely that further mutations are required for TMD to develop into AMKL. The *GATA1* mutations found in TMD and AMKL always have the same effect, causing translation to initiate at the second ATG of the coding region, leading to the production of a shorter protein, termed GATA1s. Trisomy of Hsa21 on its own, even in the absence of GATA1s, leads to an expansion of the megakaryocyte-erythroid progenitor population in fetal livers from human DS abortuses (108,109). These data suggest that trisomy of Hsa21 perturbs hematopoiesis, making megakaryocyte-erythroid progenitors susceptible to the effects of GATA1s, thereby promoting development of TMD. Several groups have reported the presence of mutations in Janus Kinase 3 (*JAK3*) in a small proportion of TMD/AMKL patients (110–115). It was suggested that *JAK3* inhibitors could be used as a therapy (111,114). However, both loss- and gain-of-function mutations have been found, so this may not be a viable treatment. Stem cell factor/*KIT* signalling has recently been demonstrated to stimulate TMD blast cell proliferation, and inhibitors of this pathway may be a treatment for severe TMD (116).

Attempts have been made to model these disorders in mice with a view to establishing which genes on Hsa21 need to be present in three copies in order to induce disease. A study of the Ts65Dn mouse model showed that it developed a late-onset myeloproliferative disorder, but did not develop leukaemia (117). It may be that the Ts65Dn model is not trisomic for the relevant dosage-sensitive genes required for the development of AMKL or that the expression of a mutant form of GATA1 will be required to increase the frequency of leukaemogenesis in this mouse model of DS.

The genetic events involved in DS-ALL are less well understood than those in DS-AMKL. A number of studies have reported DS-ALL cases with chromosomal abnormalities, gain-of-function mutations in *JAK2* and submicroscopic deletions of genes including *ETV6*, *CDKN2A* and *PAX5* (118–121).

Although the incidence of leukaemia and cancer of the testis are increased in DS, the risk of developing most solid tumours is reduced (122,123). Crossing mouse models of DS with mice heterozygous for the *Apc*^{min} mutation reduced the number of tumours, which would normally accumulate in this model of colon cancer (124). Protection against the development of tumours required three copies of the Hsa21 'proto-oncogene' *Ets2*, suggesting that in this context, *Ets2* may be acting as a tumour suppressor (124).

Hypertension

People with DS have been reported to have a reduced incidence of hypertension (125,126). Trisomy of the Hsa21 microRNA hsa-miR-155 may contribute to this (12). Hsa-miR-155

is proposed to specifically target one allele of the type-1 angiotensin II receptor (*AGTRI*) gene, resulting in its under-expression, which may contribute to a reduced risk of hypertension. Further studies are required to validate this hypothesis and determine whether other genes may also protect people with DS against hypertension.

RECENT ADVANCES IN THERAPY AND FUTURE PROSPECTS

Recent interest in therapy for people with DS has focused on pharmacological treatment to enhance cognition. A number of compounds have been shown to improve learning in the Ts65Dn mouse model. Chronic treatment with picrotoxin or pentylentetrazole improved hippocampal-based learning and LTP deficits in Ts65Dn mice, even after treatment had ceased (127). These compounds reduce gamma-aminobutyric acid-mediated inhibition in the hippocampus and are proposed to improve cognition by releasing normal learning from excess inhibition. Learning in Ts65Dn mice is also improved by the non-competitive N-methyl-D-aspartic acid receptor (NMDAR) antagonist, memantine (128). Memantine partially inhibits the opening of the NMDAR and is proposed to counter the effect of trisomy of *RCANI* on the function of the receptor. Further studies and clinical trials are required to further investigate the potential of these drugs to improve cognition in people who have DS.

To develop new therapeutic targets, it is necessary to determine the identity of genes that contribute to DS phenotypes. This requires a precise and standardized definition of phenotype. Ideally, these measurements should be formulated into a standardized protocol that can be applied at multiple centres, to permit sufficiently large numbers of samples for meaningful analysis to be collected. This can be facilitated by a carefully designed and curated biobank of detailed phenotypic data alongside DNA and tissue samples from participating individuals. These collections can then be used for both candidate gene and genome-wide analyses, by different investigators, permitting the identification of both dosage-sensitive trisomic Hsa21 and non-Hsa21 genes that contribute to DS phenotypes. Pooling of large data sets has led to recent important findings in the study of schizophrenia, diabetes and obesity, illustrating the importance of large-scale collaboration (129–132). The careful collection of additional patient data will add much to our current understanding of DS.

As recent progress demonstrates, mouse models can be used in parallel with data collected from people with DS to test genetic associations, to explore biological mechanisms and to trial therapies. In addition to the long-standing Ts65Dn and Ts1Cje models, the newly developed mouse strains such as Tc1, Dp1Yu and Ts1Rhr have generated a range of models with distinct sets of trisomic genes (Fig. 1) (19–27). Furthermore, the crossing of these strains with mice-bearing deletions of chromosomal segments syntenic to Hsa21, such as MslYah and MslRhr (Fig. 1), will allow systematic mapping and eventually identification of the dosage-sensitive genes causing DS-associated pathology.

DS was once thought to be an intractable condition because of the genetic complexity underlying it. Here, we have

described recently reported breakthroughs in the understanding of Hsa21 trisomy, illustrating that research efforts in this field are making significant strides to understand and to develop treatments for the debilitating aspects of the syndrome. Many issues vital to the health and well-being of people with DS remain to be studied, making this an important and exciting time for Hsa21 trisomy research.

ACKNOWLEDGEMENTS

We thank Roger Reeves, Dalia Kasperaviciute, Olivia Sheppard and Matilda Haas for advice on the manuscript and we thank Ray Young for help with preparation of the figures. We apologize to the many authors whose work we were unable to cite owing to space limitations.

Conflict of Interest statement. None declared.

FUNDING

V.L.J.T. and K.A.A. are funded by the UK Medical Research Council, the EU, the Leukaemia Research Fund and the Wellcome Trust; F.K.W. and E.M.C.F. are funded by the UK Medical Research Council, the Wellcome Trust and the Fidelity Foundation.

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