

# Stem and progenitor cell dysfunction in human trisomies

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## Abstract

**Trisomy 21, the commonest constitutional aneuploidy in humans, causes profound perturbation of stem and progenitor cell growth, which is both cell context dependent and developmental stage specific and mediated by complex genetic mechanisms beyond increased Hsa21 gene dosage. While proliferation of fetal hematopoietic and testicular stem/progenitors is increased and may underlie increased susceptibility to childhood leukemia and testicular cancer, fetal stem/progenitor proliferation in other tissues is markedly impaired leading to the characteristic craniofacial, neurocognitive and cardiac features in individuals with Down syndrome. After birth, trisomy 21-mediated premature aging of stem/progenitor cells may contribute to the progressive multi-system deterioration, including development of Alzheimer's disease.**

**Keywords** Down syndrome; hematopoietic stem cells; leukemia; neural progenitors; trisomy 21

**DOI** 10.15252/embr.201439583 | Received 14 September 2014 | Revised 16 November 2014 | Accepted 20 November 2014

**EMBO Reports (2015) 16: 44–62**

See the Glossary for abbreviations used in this article.

## Introduction

Trisomy 21, trisomy 18 and trisomy 13 are the commonest constitutional trisomies in humans [1]. In contrast to trisomy 18 and 13, where fewer than 10% of affected children survive beyond the first year of life [2–4], median life expectancy for individuals with trisomy 21 (Down syndrome; DS) is around 60 years [5]. Most attention has focused on trisomy 21, not only because it is 20 times and 40 times more frequent than trisomy 18 and 13, respectively, but also because prolonged survival in DS suggests that most cells evolve epigenetic, transcriptional and/or translational regulatory mechanisms which allow them to adapt to the additional copy of chromosome 21 (Hsa21). To some extent, this may reflect the relatively low number of protein-encoding genes on Hsa21 (~240) (<http://www.ensembl.org/biomart/martview/>). However,

the characteristic phenotypic variability between different individuals with DS points to considerable complexity. Understanding the genomic determinants of this complexity continues to reveal fascinating insights relevant not only to DS, but also to aneuploidy in general.

Here, we review the impact of human trisomies on stem and progenitor cells. We will focus on trisomy 21, and particularly on hematopoiesis, where advances in techniques for characterization of highly purified primary cells and the development of induced pluripotent stem cell (iPSC) and animal models are beginning to answer some of the questions about the mechanisms by which trisomies cause human disease.

## Phenotypic variability in constitutional trisomy 21 (DS)

DS is a multisystem disorder caused, in most cases, by meiotic non-disjunction of the maternal Hsa21, resulting in a third copy of the entire Hsa21 in all cells [6,7]. The clinical and biological impact of trisomy 21 nevertheless varies widely, not only between individuals with DS, but also in different tissues, the cell types within these tissues and at different ages [reviewed in 8–10]. Within this phenotypic variability, certain characteristics, such as the craniofacial abnormalities, hypotonia and cognitive impairment, are common to all individuals with DS (Table 1) and may therefore share temporal, biological or genetic mechanisms. Other features, such as cardiac defects or gastrointestinal anomalies, affect only a subset of patients and so may be more strongly influenced by inter-individual differences which interact with trisomy 21-driven changes, in heart and gut development, respectively, during embryogenesis. Many of these phenotypic abnormalities can be modeled using mouse segmental trisomies allowing the consequences of trisomy 21 to be investigated in an appropriate cellular context (Table 2).

The impact of age on the phenotypic expression of DS is increasingly recognized and, for many cells and tissues, is essential to consider in selecting the best experimental model to investigate the role of trisomy 21. Abnormalities of hematopoiesis begin in fetal life and have their maximal expression in the neonatal period when nearly all DS neonates have multiple hematologic defects, including 30% who develop a unique preleukemic syndrome confined to the first few months of life [11]. By contrast, the effects of trisomy 21 on

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**Glossary**

<b>AICD</b>	APP intracellular domain
<b>ALL</b>	acute lymphoblastic leukemia
<b>AVSD</b>	atrioventricular septal defect
<b>BCP</b>	B-cell progenitor
<b>DS</b>	Down syndrome
<b>FDR</b>	false discovery rate
<b>GEDD</b>	gene expression dysregulation domain
<b>GEP</b>	gene expression profile
<b>GMP</b>	granulocyte–monocyte progenitor
<b>GSK3b</b>	glycogen synthase kinase 3b
<b>hESC</b>	human embryonic stem cell
<b>Hsa21</b>	<i>Homo sapiens</i> chromosome 21
<b>HSC</b>	hematopoietic stem cell
<b>HSPC</b>	hematopoietic stem and progenitor cell
<b>IGF</b>	insulin-like growth factor
<b>iPSC</b>	induced pluripotent stem cell
<b>MEP</b>	megakaryocyte–erythroid progenitor
<b>miR</b>	microRNA
<b>ML-DS</b>	myeloid leukemia of Down syndrome
<b>Mmu</b>	<i>Mus musculus</i>
<b>NFAT</b>	nuclear factor of activated T cells
<b>qPCR</b>	quantitative polymerase chain reaction
<b>Shh</b>	sonic hedgehog
<b>T21</b>	trisomy 21
<b>TAM</b>	transient abnormal myelopoiesis
<b>VEGF</b>	vascular endothelial growth factor

visual and hearing impairment, thyroid function and cognitive function increase with age with progressive pathological changes in the brain in almost all DS individuals and clinical evidence of dementia in ~50% [12–19]. These age-related differences in phenotypic expression in DS suggest that trisomy 21, through patterns of gene expression which may be established early in development, causes premature, or accelerated, aging of a range of cell types. Evidence in support of this is now emerging [20], as discussed below.

Although several Hsa21 genes have been linked to the phenotypic expression of specific aspects of DS, such as leukemia and dementia, the mechanism(s) by which trisomy of individual genes or groups of genes contributes to the disorder remains unclear [reviewed in 8,9,21–24]. Investigators have used three broad approaches to investigate this question: mouse models trisomic for one or more of the genes on Hsa21, genomic association studies and comparative studies between human cells trisomic or disomic for Hsa21.

### Mouse models of DS

The phenotypic characteristics of the most well-established mouse models of DS, and the extent to which they recapitulate the human phenotype, are summarized in Table 2. These include the only transchromosomal mouse model (Tc1) in which most of Hsa21 is present [25] albeit with several deleted or rearranged genes [26]. A number of more recent mouse mutants carrying genomic rearrangements of Hsa21 syntenic regions (on Mmu10, Mmu16 and Mmu17) that are trisomic for some, or most, of the ~250 mouse genes orthologous to Hsa21 genes have been described [27–31]. These interesting models, which may better mimic some aspects of human DS, have so far been used mainly to model the neurocognitive and

cardiac defects in DS [27–31]. Details of these, and of elegant refinements to narrow down the Hsa21 regions linked to defined phenotypes, are described in several reviews [32–42] and are only briefly discussed here in relation to their insight into the effects of trisomy 21 on stem/progenitor cells.

### The impact of trisomy 21 on stem cell function

There is increasing recognition that trisomy 21 impacts on stem cell function in a number of ways (Fig 1). In hematopoiesis, for example, trisomy 21 affects the self-renewal, proliferation and differentiation of hematopoietic stem and progenitor cells (HSPC) either directly or via the hematopoietic microenvironment [43–52]. Studies in other tissue types, where stem and progenitor cells are often more difficult to identify and isolate, suggest that trisomy 21 also causes many of the defects in craniofacial, brain and cardiac development through perturbations of stem/progenitor cell growth and differentiation and altered interactions with microenvironmental and temporal cues. These alterations in stem/progenitor proliferation may underlie the increased susceptibility of some cell types, such as HSPC and primordial germ cells to malignant transformation [53–60] and of HSPC to premature aging in DS [20], as discussed in detail below.

#### Hematopoiesis and leukemia

The link between childhood leukemia and DS provides strong evidence for a particular susceptibility of hematopoietic cells early in life to perturbation of the normal mechanisms which control their growth and differentiation. Leukemias in DS have several unique features which hint at the ways in which trisomy 21 alters the behavior of HSPC [reviewed in 21,61,62]. First, the frequency of both myeloid leukemias and lymphoid leukemias is increased, by 150-fold and ~30-fold, respectively [53,59], indicating that trisomy 21 affects both myeloid and lymphoid progenitors. Second, these leukemias have a distinct temporal pattern of onset. Myeloid leukemia of DS (ML-DS) originates in fetal liver HSPC and presents either as a neonatal preleukemic syndrome known as transient abnormal myelopoiesis (TAM) or as full-blown ML-DS in children under the age of 5 years [21,61,62]. The peak age at presentation for acute lymphoblastic leukemia in DS (DS-ALL) is 1–4 years and, in contrast to ALL in individuals without DS, never presents in neonates or infants [53]. Third, leukemias in DS have distinct biologic and molecular features. Leukemic cells in ML-DS and TAM harbor N-terminal truncating mutations in the key hematopoietic transcription factor *GATA1*, which result in exclusive production of a short *GATA1* protein (Gata1s) with altered functional properties together with loss of expression of full-length *Gata1* since the *GATA1* gene is on the X chromosome [63–67]. Such mutations are not leukemogenic in the absence of trisomy 21 [68]. In DS-ALL, which in contrast to ALL in children without DS is always a B-precursor disease [69], ~60% of cases have aberrant expression of the CRLF2 receptor and around half of these have *RAS* mutations or mutations activating JAK-STAT growth-promoting signaling pathways [70–76].

**Impact of trisomy 21 on fetal, neonatal and adult human hematopoiesis** In contrast to most other tissues, hematopoietic tissues

**Table 1. Phenotypic characteristics of Down syndrome.**

Characteristic	Frequency (%)	Reference
Craniofacial Epicanthal folds Upward slanting palpebral fissures Flat nasal bridge Small brachycephalic head Small ears Small mouth	~100	[10, 114, 194, 195]
Other musculoskeletal abnormalities Hypotonia Single transverse palmar crease Clinodactyly with wide spacing	~100	[10, 196]
Cognitive impairment Reduced brain volume Learning and memory defects	~100	[10, 17, 197]
Dementia	40–50, increases with age	[12, 13, 14, 15, 16, 17, 18, 198, 199]
Visual	18–60, increase with age	[10, 18]
Hearing	18–80, increase with age	[10, 18]
Thyroid disease	1–54, increase with age	[10, 18, 196, 200, 201]
Cardiac defects ASD (45%) VSD (35%) Isolated secundum (8%) Isolated PDA (7%) Isolated Fallot's (4%) Other	40–50	[202, 203, 204]
Gastrointestinal defects	12	[10]
Benign hematological abnormalities Neonatal thrombocytopenia Neonatal polycythemia Neonatal neutrophilia, blast cells Macrocytosis	~100	[11]
Preleukemia and leukemia TAM and silent TAM Acute myeloid leukemia Acute lymphoblastic leukemia	30 1 1	[11, 53] [53] [53]
Non-hematologic cancers	50% of risk of individuals without DS	[18, 53, 56, 58, 59]

contain a well-characterized hierarchy of stem and progenitor cells, which can be readily isolated for molecular and functional studies. Characterization of the hematologic abnormalities in human DS therefore offers one of the best ways to understand how trisomy 21 perturbs cell biology and how cells adapt to aneuploidy. Recent studies in primary human fetal liver and neonatal cells [11,45], supported by data from human iPSC and hESC [46,47], demonstrate that trisomy 21 causes major disturbance throughout the entire hematopoietic hierarchy from HSC through to progenitors and mature cells (Fig 2). In particular, in fetal liver, trisomy 21 alters the balance of HSPC differentiation, promoting expansion and proliferation of megakaryocyte–erythroid progenitors (MEP) and megakaryocytes during the second trimester at the expense of both granulocyte–monocyte progenitors (GMP) and B-cell progenitors (BCP) [45]. There is also a 3.5-fold expansion in HSC numbers, and *in vitro* purified trisomy 21 fetal liver HSCs have erythroid–megakaryocyte-biased gene expression together with reduced

expression of lymphoid genes. Consistent with this, fetal liver HSC function is also markedly abnormal in DS. In particular, fetal liver HSCs generate more megakaryocyte and erythroid cells while their B-cell potential is severely impaired [45]. Since *GATA1* mutations were not detectable in these cells, these data indicate that trisomy 21 itself perturbs fetal liver hematopoiesis.

The effects of trisomy 21 on primary human fetal liver HSPC raise many questions. First, since these studies were confined to second-trimester fetal liver, it is not clear whether the effects are confined to this gestation. Interestingly, Chou *et al* [47] found that trisomy 21 iPSC differentiated under conditions designed to model yolk sac hematopoiesis showed enhanced erythroid, but not megakaryocyte, differentiation *in vitro*, suggesting the effects of trisomy 21 may be developmental stage specific. More recently, our group studied hematopoiesis in neonates with DS. In the presence of *GATA1* mutations, DS neonates developed the preleukemic condition, TAM. However, even in the absence of *GATA1* mutations, DS

Table 2. Mouse models of Down syndrome.

	Ts65Dn	Ts1Cje	Ts1Rhr	Tc1	Ts1Yeh;Ts2Yeh;Ts3Yeh Dp(16)1Yeh/+; Dp(10)1Yeh/+; Dp(17)1Yeh/+	Ts1Yah
Number of trisomic Hsa21 orthologs	~100 (also trisomic for ~60 genes on Mimu17 not syntenic for Hsa21)	~80	33	Transchromosomic (trisomic for 200 RefSeq Hsa21 genes*)	~175	~12
Craniofacial	Small mandible Brachycephaly	Small mandible	Large mandible	Small mandible	Normal appearance	Not reported
Learning and memory	Differences in face, palate-recapitulate human DS Impaired spatial learning and memory Impaired motor coordination	Differences in face, palate-recapitulate human DS Altered hippocampal dependent learning	Abnormalities do not recapitulate human DS Impaired novel object recognition	Defect in short-term memory and motor co-ordination	Recapitulates most of the behavioral features of Ts65Dn	Impaired novel object recognition but improved hippocampal-dependent spatial learning
Brain structure	Reduced brain volume Reduced cerebellar volume	Reduced brain volume during embryogenesis Reduced cerebellar volume	Reduced brain volume at age 4 months Reduced cerebellar volume	Reduced cerebellar volume	Hydrocephalus (6.5%)	Not reported
Cardiac defects	Septal defects similar, but not identical, to human DS	Not reported	None	Mainly VSD; also outflow tract defects and AVSD similar to human DS	Cardiac defects include ASD VSD and AVSD (in several models: Dp(16)1Yeh/+, Dp(16)2Yeh/+, Dp(16)4Yeh/+ and Dp(16)1Yeh/+; Dp (16)2Yeh/+; Dp(16)3Yeh/+	Not reported
Gastro-intestinal defects	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Thyroid disease	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Hematopoietic	MPD in adults No leukemia Macrocytosis Reduced adult HSC Impaired HSC self-renewal in adults	No MPD or leukemia Macrocytic anemia Normal HSC numbers and function in adults Impaired fetal liver HSC and progenitor function	Co-operates with GATA1s and MPL to induce AMKL Thrombocytosis, increased MKs and mild anemia in adults	No MPD or leukemia even with GATA1s Macrocytosis Increased MKs and erythrocytosis in older adults	Not reported	Not reported
	Increased GMP in adults	Increased GMP in adults	Increased GMP in adults			

AMKL, acute megakaryocytic leukemia; ASD, atrioseptal defect; AVSD, atrioventricular septal defect; DS, Down syndrome; GMP, granulocyte-macrophage progenitor; HSC, hematopoietic stem cell; MPD, myeloproliferative disorder; VSD, ventriculoseptal defect. See text for details.

neonates had trilineage perturbation of hematopoiesis with increased erythroid and myeloid cells and abnormal platelet development consistent with the effects of trisomy 21 on HSPC function persisting after birth [11]. In contrast, the few studies in adults with DS suggest that trisomy 21 causes a different profile of hematologic abnormalities later in life. Adults with DS have a high prevalence of red cell macrocytosis and quantitative and qualitative B- and T-lymphocyte abnormalities, while some have unexplained thrombocytopenia and neutropenia [77–79], myelodysplasia or bone marrow failure [80]. This suggests that in adults, trisomy 21 may induce HSC aging, consistent with recent studies in Ts65Dn mice implicating increased expression *USP16* as a possible mechanism for these effects [20].

Second, the mechanisms linking trisomy 21-mediated perturbation of fetal liver hematopoiesis and the high frequency of *GATA1* mutations in DS neonates are still unclear. Trisomy 21-mediated proliferation of fetal liver megakaryocyte/erythroid-biased HSPC may simply provide a permissive cellular environment for expansion of preleukemic mutant *GATA1* clones. Alternatively, changes to pathways regulating fetal HSPC growth and differentiation in DS may be directly responsible for the increased frequency of *GATA1* mutations. Similarly, the link between impaired B-cell development in DS fetal liver HSPC and the increase in B-ALL [69,81,82] and of immune deficiency in children with DS [83] is unclear, although delayed expression of the normal fetal B-cell development program might increase the likelihood of acquiring leukemogenic mutations in lymphoid genes in early childhood.

Third, given that alterations in the microenvironment can promote myeloproliferative disorders and leukemia in mouse models [84–87], the DS fetal liver microenvironment may support, or even drive, the abnormal growth and differentiation of DS fetal liver HSPC. The natural history of TAM, which resolves within a few weeks of life in most cases and is characterized by infiltration of the liver by mutant *GATA1* blast cells [88,89], also suggests that factors produced in the fetal liver microenvironment may be necessary to maintain these cells. In support of this, *in vitro* survival of TAM blast cells and *in vivo* survival of leukemia cells in a mouse model of DS-like acute myeloid leukemia has been shown to be dependent on insulin-like growth factors [90].

Finally, the molecular basis for perturbation of fetal liver HSPC growth and differentiation by trisomy 21 remains to be explained. Even using highly purified fetal liver HSPC, we found no significant increase in expression of selected trisomic genes on Hsa21 (*RUNX1*, *ERG*, *DYRK1A*) known to influence HSPC behavior and development of leukemia through gene dosage [51,91]. This does not exclude a role for trisomy 21 dose-related changes in these genes given limitations in the sensitivity of the methodology [92] and the confounding influence of interindividual variation [93] as discussed below, especially since even small changes in expression of the Hsa21 genes are associated with DS-like defects in mouse models [94] and multiple genes may be involved [95].

#### Animal models of leukemia and abnormal hematopoiesis in DS

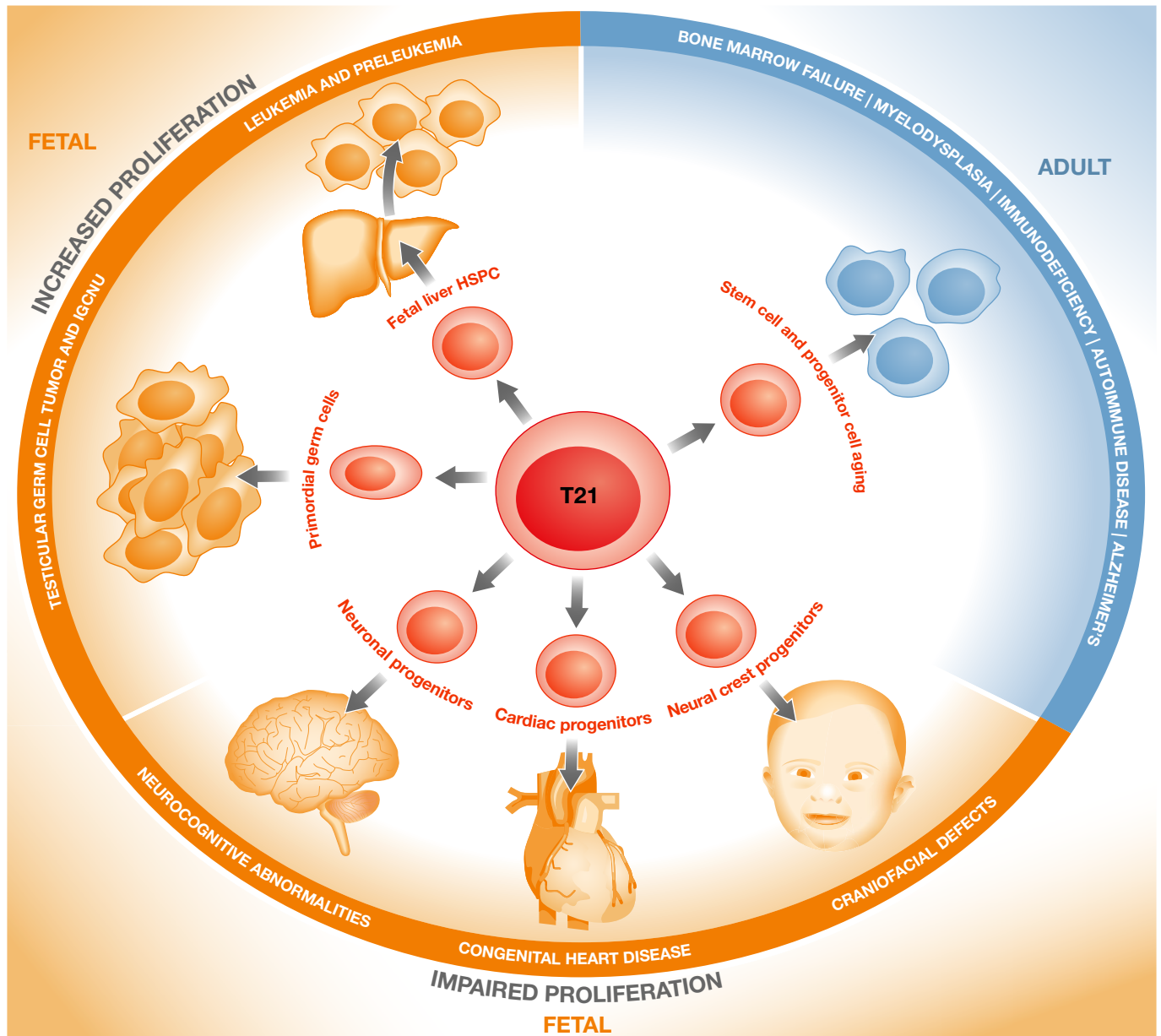
Although ML-DS and TAM provide a natural human model to interrogate the impact of trisomy 21 on HSPC and the mechanisms which contribute to the development of leukemia in DS, mechanistic experiments to identify the exact role of specific genes are often difficult in human cells. Initial attempts to model ML-DS and TAM

in mouse models were disappointing as no spontaneous leukemias developed (Table 2). However, this is consistent with human DS where trisomy 21 dysregulates HSPC proliferation and differentiation but is insufficient to promote leukemia without additional, acquired mutations. All of the DS mouse models have abnormal hematopoiesis, typically affecting erythroid and megakaryocyte development [48–50], although the defects do not accurately recapitulate those seen in human fetal liver [45]. Nevertheless, by co-expressing additional oncogenes, DS mouse models provide potential insight into genes and pathways, which may contribute to perturbation of HSPC development by trisomy 21, including *ERG*, *DYRK1A*, *HMG1* and *miR125b* [51,91,96–98].

The myeloproliferative disorder in adult Ts65Dn mice [48], for example, is clearly linked to gene dosage of *ERG* since reducing the number of copies of *ERG* from 3 to 2 in this model corrects the hematologic abnormalities [91]. Since neonatal TsDn mice are not affected, Birger *et al* [97] used a different approach to modeling TAM. Building on data showing potent effects of *ERG* overexpression on megakaryocyte proliferation and leukemia in disomic mice [100], they recently created a double transgenic mouse model of TAM/ML-DS on a non-trisomic background in which overexpression of *ERG* promoted fetal liver MEP expansion similar to that seen in human fetal liver, and this synergized *in vivo* with expression of *GATA1s* to cause a TAM-like disease and subsequent progression to megakaryocyte–erythroid leukemia [97]. Nevertheless, *ERG* has not yet been shown to be significantly overexpressed in trisomy 21-containing human hematopoietic cells, including leukemias [45,100] and hESC/iPSC [46,47].

Malinge *et al* [51] recently used Ts1Rhr mice, trisomic for 33 Hsa21 orthologs (Table 2), to create a trisomy 21-dependent ML-DS model by crossing them with *GATA1s* knock-in mice and overexpressing a transforming *MPL* allele (*MPL*<sup>W515L</sup>), which has been reported in ML-DS [101,102]. In this model, they showed that *DYRK1A* was able to act as a megakaryoblastic tumor-promoting gene and they found increased expression of *DYRK1A* in human ML-DS samples [51]. Although this identifies a possible role for increased expression of *DYRK1A* in the transformation of TAM to ML-DS, this model does not fully recapitulate the human disease. For reasons that are still not clear, this model can only be produced in adult, and not fetal, hematopoietic cells, and indeed, *DYRK1A* expression does not appear to be significantly increased in human fetal HSPC [45], perhaps indicating altered mechanisms of *DYRK1A* regulation in fetal cells compared to postnatal or leukemic cells. Furthermore, *MPL*<sup>W515L</sup> is able to induce a fatal, rapid onset myeloproliferative disorder even in the absence of *Gata1s* and a trisomic background [103] highlighting the importance of the cellular context in understanding the contribution of individual genes.

The Ts1Rhr mouse model has also proved useful for investigating the role of Hsa21 orthologs in B-cell development and B-ALL. Lane *et al* [98] recently showed that, as in human fetal liver [45], B progenitors were reduced in bone marrow from young Ts1Rhr mice but were more clonogenic than wild-type progenitors and could be replated indefinitely *in vitro*. Furthermore, Ts1Rhr B progenitors were transformed into B-ALL *in vivo* by *CRLF2* with activated *JAK2*, a known oncogenic stimulus in DS-ALL. Lane *et al* then identified differential expression of *PRC2* targets and sites of H3 K27 trimethylation as a specific ‘signature’ common to DS-ALL and

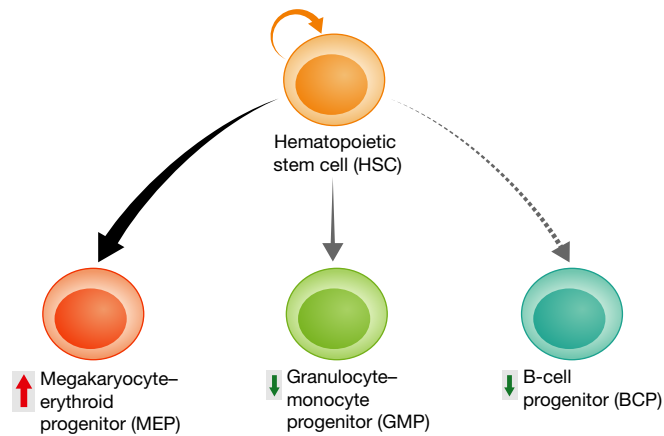


**Figure 1. Impact of trisomy 21 on stem and progenitor cell function.**

Studies in human cells and in animal models of Down syndrome (DS) show that trisomy 21 can affect the self-renewal, proliferation and differentiation of stem and progenitor cells either directly or via the supportive microenvironment. In fetal life in DS, proliferation of hematopoietic and testicular stem/progenitor cells is increased and susceptibility to malignant transformation (leukemia and testicular cancer) is increased in childhood. By contrast, proliferation of stem/progenitor cells of other lineages is impaired and is responsible for many of the developmental defects affecting the brain, craniofacial structures and heart in DS. After birth, trisomy 21 has been shown to cause premature aging of stem and progenitor cells both of hematopoietic and non-hematopoietic lineages, an effect which is likely to contribute to the phenotypic abnormalities in adults with DS, including Alzheimer's disease, bone marrow failure and impaired immunity.

Ts1Rhr B cells and, through a series of elegant experiments, showed that overexpression of *HMGN1*, an Hsa21 ortholog trisomic in Ts1Rhr mice which encodes a nucleosome remodeling protein, is responsible both for this gene expression signature and for the proliferative and leukemia-promoting effects on Ts1Rhr B cells. These data provide compelling evidence in support of a role for *HMGN1* in the perturbation of B-cell development by trisomy 21 and the increased susceptibility of children with DS to B-ALL.

**USP16 and defects in HSC self-renewal and stem cell aging** By comparing hematopoiesis in Ts65Dn, Ts1Cje and wild-type mice, Adorno *et al* [20] identified a role for the mouse homolog of the Hsa21 gene *USP16* in HSC self-renewal. HSC frequency was reduced by greater than threefold in Ts65Dn mice, which are trisomic for *USP16*, compared to Ts1Cje mice and wild-type mice, which have only 2 copies of *USP16*. HSC function was also impaired in the *USP16* trisomic mice with reduced clonogenicity and multilineage



**Figure 2. Perturbation of human fetal hematopoiesis by trisomy 21.**

Comparison of the frequency and function of disomic and trisomy 21 second-trimester human fetal hematopoietic stem cells and progenitor cells (HSPC) has shown a consistent pattern of abnormalities in the trisomic populations. Trisomy 21 alters the balance of HSPC differentiation, promoting expansion and proliferation of megakaryocyte–erythroid progenitors (MEP) and megakaryocytes during the second trimester at the expense of both granulocyte–monocyte progenitors (GMP) and B-cell progenitors (BCP), which are both significantly reduced.

engraftment following secondary transplantation. These features were associated with a 1.5-fold increase in *USP16* gene expression and were reversed by short interfering RNAs. Interestingly, similar defects were seen in Ts65Dn neural progenitors and fibroblasts consistent with previously reported defective proliferation of primary human DS fibroblasts [104,105]. They also went on to demonstrate a link between trisomy for *USP16* and reduced activity of the PRC1 complex and its target *CDK2NA*, which regulate senescence and self-renewal of several somatic stem cell types [106,107; reviewed in 108]. The reduction in HSC frequency and clonogenicity contrasts with the increase in HSC frequency and clonogenicity in human DS fetal liver [45]. However, although this may reflect species-specific differences in hematopoiesis and/or the role played by other genes/pathways in the senescence of Ts65Dn mouse HSC, another important issue is age. The impaired HSC self-renewal reported by Adorno *et al* in adult Ts65Dn mice [20] is compatible with the increasing recognition of the occurrence of hematologic abnormalities, including myelodysplasia and bone marrow failure, in older adults with DS [80].

#### Non-hematologic cancers

It is likely that several mechanisms contribute to the 50% reduction in the frequency of solid tumors with DS, including the effects of trisomy 21 on stem and progenitor proliferation, tumor-associated angiogenesis and tumor suppression [reviewed in 22]. It is notable that the only malignancy, apart from leukemia, to be increased in DS is testicular germ cell tumors. These tumors are derived from primordial germ cells and, in DS, are believed to arise *in utero* through a pre-invasive stage known as intratubular germ cell neoplasia unclassified (IGCNU), which has been documented in the second trimester [109,110] and which is preceded by activation of signaling pathways leading to increased proliferation and impaired differentiation [60]. These cellular abnormalities are similar to those

in fetal HSPC, and, interestingly, testicular germ cell tumors also share several key signaling pathways, such as *KIT/SCF*, *K-RAS* and *PZLF* with normal and leukemic HSPC [60] and suggesting that increased susceptibility to both these malignancies in DS may derive from the trisomy 21-mediated proliferative drive to stem cells within fetal hematopoietic and testicular tissues.

Investigations into the mechanisms of reduced tumor susceptibility in DS, largely through studies in mouse models, suggest that 3 Hsa21 genes, *ETS2*, *RCAN1* and *DYRK1A*, may play a role. Through crossing Ts1Rhr mice with *Apc<sup>Min</sup>* mice, which are heterozygous for the adenomatosis polyposis coli gene, and *Ets2<sup>+/-</sup>* mice, Sussan *et al* [111] showed that protection against colonic tumors in this model was in part dependent on the presence of three copies of *ETS2*. This suggests that *ETS2* can act as a tumor suppressor, through as yet unclear mechanisms, but that other genes may contribute to this effect. Subsequently, Baek *et al* [112] used the Ts65Dn mouse model and a transgenic disomic mouse over-expressing *RCAN1* to show that increased expression of *RCAN1* was sufficient to suppress growth of lung cancer and melanoma cell lines *in vivo* through inhibition of VEGF-mediated tumor angiogenesis by suppressing the calcineurin pathway in co-operation with *DYRK1A*. However, more recent experiments in a more aggressive tumor model (NPcis) found that trisomy improved survival rather than preventing cancer and that neither *ETS2* nor tumor angiogenesis was responsible for this protective effect [113]. Taken together, these studies indicate that the mechanisms underlying tumorigenesis in DS result from a complex interplay between changes in expression of Hsa21 and other genes, inter-individual differences in genetic susceptibility and acquired changes in the microenvironment.

#### Craniofacial defects

In contrast to the hematopoietic system, there is limited information about the effects of trisomy 21 on the stem and progenitor cells involved in craniofacial development during fetal life, especially in humans. Most of the insight into the mechanisms by which trisomy 21 causes the craniofacial defects in DS has relied on animal models. Using detailed imaging, Richtsmeier *et al* [114] have shown that the characteristic craniofacial defects in individuals with DS are closely mimicked early in development in Ts65Dn mice and, to a slightly lesser extent, in Ts1Cje mice [115]. In particular, there is hypoplasia of the mandible and mid-facial skeleton [114], structures which in normal mouse development are known to be derived from cells which migrate from the cranial neural crest to populate the craniofacial precursors of the mid and lower face [reviewed in 116]. Using Ts65Dn mice crossed to mice expressing lacZ under the control of the *Wnt1* promoter, Roper *et al* have made a number of important observations about mechanism of these DS-associated craniofacial abnormalities. First, they demonstrated that the number of neural crest cells was significantly reduced in trisomic compared to control (euploid) embryos [117]. Second, they showed that this was due both to reduced generation of neural crest cells and to impaired migration into the first pharyngeal arch (PA1), which goes on to form the maxilla and lower jaw. They then found that *in vitro* proliferation of these trisomic PA1-derived cells in short-term culture was reduced compared to euploid controls. The defect in proliferation of the PA1 cells was partially rescued by addition of the mitogen Sonic Hedgehog (Shh), suggesting that the

defects in neural crest generation and proliferation might be due, at least in part, to impaired Shh responsiveness [117]. This is interesting because, as discussed below, abnormal Shh signaling is also implicated in the reduced proliferation of cerebellar granule precursors in the Ts65Dn mouse, although whether this is directly or indirectly linked to a specific trisomic gene(s) is not yet clear [118,119].

To identify genes and pathways which underlie the defects in PA1 neural crest cells, Billingsley *et al* [120] isolated mandibular precursor cells from embryonic day 13.5 (E13.5) Ts65Dn mice and compared their gene expression with the same cell population isolated from euploid controls using microarray. Of the relatively small number of differentially expressed genes, 20 contained homeobox DNA-binding domains, including increased expression of at least two genes (*EN2* and *OTX2*) reported to have a role in mandibular development [121,122], reduced expression of all 12 of the differentially expressed HOX genes and a modest increase (1.2-fold) in expression of *SOX9*, known to be important for normal skeletal development [120]. The extent to which these changes in gene expression are linked to the craniofacial defects in Ts65Dn mice and how they are linked to trisomy is an intriguing puzzle which remains to be investigated particularly given that expression of Ts65Dn trisomic genes in the mandibular precursor cells was not increased compared to euploid controls.

The most studied candidate genes on Hsa21 linked to the craniofacial abnormalities in DS are *DYRK1A*, *RCAN1* (*DSCR1*) and *ETS2* [94,123–125]. Arron *et al* noted the similarity between the craniofacial defects in calcineurin-deficient and Nfatc-deficient mice and those seen in DS and went on to show that *DYRK1A* and *RCAN1* can act synergistically to prevent activation of NFATc-target genes and would therefore be plausible mediators of the craniofacial defects in DS. However, the role of *DYRK1A* and *RCAN1* in craniofacial development was not directly addressed in this study, and therefore, the extent to which perturbed NFATc-signaling due to increased *DYRK1A/RCAN1* expression contributes to craniofacial defects in DS remains unclear [94]. Studies in DS mouse models have more directly addressed the role of *DYRK1A*, *RCAN1* and of *ETS2* in the craniofacial defects [112,123,126–128]. Taken together, these studies suggest that trisomy of each of these genes individually is insufficient to cause the characteristic DS-associated craniofacial phenotype.

It is clear that interpreting the impact on craniofacial development of differences in expression of individual genes in DS mouse models is extremely difficult and needs to take into account differing mouse genetic backgrounds, as well as developmental stage, cellular context and interactions between other trisomic and non-trisomic genes [23]. As in primary human fetal hematopoietic cells, perturbation of craniofacial development by trisomy 21 may be largely mediated via non-trisomic genes and/or by small changes in the level of expression of multiple trisomic genes which are difficult to detect using standard methods. Nevertheless, the close match between the Ts65Dn mouse and human phenotype, the ability to alter copy number of individual genes or groups of genes in this model and the identification of the relevant stem/progenitor cells now provide crucial tools to investigate candidate genes in DS craniofacial defects and the mechanisms by which they are linked to trisomy 21.

#### Abnormalities of brain structure and function

Studies in individuals with DS and in DS mouse models indicate that intellectual disability in DS is directly related to impaired development

of many areas of the brain, including the cerebellum, the visual, auditory and somatosensory cortex, the motor cortex and the superior temporal gyrus [129]. Many of the available DS mouse models recapitulate the structural and functional brain abnormalities of human DS very closely [reviewed in 24,32,39,130]. Here, we briefly discuss recent studies which have investigated the cellular and genomic basis for these defects.

The most consistent finding, both in DS animal models and in primary human samples, is of reduced cell numbers in several specific areas of the brain [119,126,129–136]. In second-trimester human DS fetal brain, a number of studies have shown that total cell numbers are reduced in the hippocampus, dentate gyrus, parahippocampal gyrus [131,132] and cerebellum [133]. Importantly, there is a particular reduction in neuronal precursor cells while astrocytic cells are preserved [132]. Assessment of the proliferative status of these cells using immunohistochemical staining for the cell cycle-associated marker Ki-67 suggests that there are fewer proliferating cells in these regions of the brain in DS samples [131–133] compared to controls together with a higher frequency of apoptotic cell death in some areas [132]. Detailed functional studies and characterization of the stem and progenitor cells populations have not yet proved possible in these tissues. However, the findings suggest that trisomy 21 causes impaired neurogenesis in DS from early in fetal development and may also affect cell fate specification (from neurones to astrocytes).

Studies in DS mouse models support the observations in human brain. Several groups have shown that neurogenesis is impaired in several areas of the brain including the hippocampus, neocortex, dentate gyrus and cerebellum and that many of these changes begin during fetal or early postnatal development [118,119,129,134–136]. Histological studies in Ts65Dn mice indicate that there are reduced numbers of mitotic cells compared to euploid mice [119,135], and more recently, administration of BrdU confirms reduced proliferation of cells in the same areas of the brain [137]. There is good evidence, both from *in vivo* studies and *in vitro* culture of neural precursor cells [118,129], of altered cell fate specification as a result of which, as in human fetal brain, the reduction in neurogenesis is accompanied by an increase in astrogliogenesis [127,131–133,136–138].

Insight into mechanisms of impaired neurogenesis has come mainly from investigation of pathways known to be important for normal neurogenesis, such as Shh, and from specific investigation of candidate genes on Hsa21, including *DYRK1A*, *RCAN1*, *GRIK2* and *APP* [118,139,140]. In particular, recent studies report dramatic improvements in neurogenesis in response to pharmacological agents, thereby implicating defects in the pathways they target in the pathogenesis of the cognitive defects in DS [141–143].

Several lines of evidence link abnormalities in the Shh pathway to the defects in neurogenesis in DS. First, cerebellar granule cell precursors isolated from the Ts65Dn DS mouse model have reduced *in vitro* responsiveness to Shh [119], which is known to be a potent mitogen for normal granule cell precursors [144]. Second, administration of a Shh agonist (SAG-1) to neonatal Ts65Dn mice restores cerebellar development to normal in adult mice and improves learning and memory [141], supporting a significant role for the Shh pathway in the pathogenesis of DS-associated cognitive defects. These data are particularly interesting given the putative role of Shh in the craniofacial defects



in DS [117,118]. Although no direct link between Shh and trisomy 21 was established in those studies, clues to the role of trisomy 21 may lie with studies into the role of the Hsa21 gene *APP*. Triplication of *APP* in Ts65Dn impairs neuron precursor proliferation, differentiation and maturation [118,129]. These effects are dependent upon the APP intracellular domain (AICD), and increased levels of AICD lead to increased transcription of the *Ptch* gene, leading to dysregulation of the Shh pathway [118]. AICD may also be involved in another pathway important in the impaired neurogenesis in Ts65Dn mice by directly interacting with, and increasing the activity of, glycogen synthase kinase 3b (GSK3 $\beta$ ), a key negative regulator of neuron proliferation, differentiation, maturation and migration [145]. Trazzi *et al* [118] recently showed that treatment of Ts65Dn mice with lithium, a GSK3 $\beta$  inhibitor, normalized neural precursor proliferation, cell fate specification and maturation, suggesting that dysregulation of the GSK3 $\beta$  signaling pathway, potentially by the AICD of APP, also plays a significant role in the impaired neurogenesis typical of DS. Interestingly, fluoxetine, a 5-HT1A receptor agonist, has also recently been shown to improve neurogenesis in the Ts65Dn mouse model. Fluoxetine increased total and proliferating neural progenitor cells, corrected defective 5-HT1A receptor expression and rescued defects in contextual memory and behavior typical of DS both in fetal [142,143] and adult Ts65Dn mice [137,143,146]. These responses may be due to direct effects of fluoxetine on the serotonergic system. However, as Trazzi *et al* [143] showed that activation of 5-HT1A receptors by fluoxetine inhibits GSK3 $\beta$ , this would provide a mechanistic link to Hsa21 (inhibition of the *APP*-driven increase in GSK3 $\beta$  activity) for the beneficial effects of fluoxetine on neurogenesis, behavior and memory in DS.

Comparison of the defects in Ts65Dn and Ts1Rhr mice (Table 2), which are trisomic for only 33 Hsa21 orthologs, with a mouse model monosomic for these genes (Ms1Rhr) identified *DYRK1A*, *GIRK2* and *SIM2* as necessary, but not sufficient, for hippocampal-based learning deficits in Ts65Dn [140]. Several lines of evidence support a role for increased expression of *DYRK1A* in DS-associated cognitive defects [125,139,147–151]. In disomic transgenic mice, overexpressing *DYRK1A* by 1.5–2-fold in cortical neurons leads to impaired neural progenitor differentiation and motor and cognitive defects [125] which are ameliorated by selective *DYRK1A* knockdown [151]. More recently, Altafaj *et al* [139] showed that *in vivo* knockdown of *DYRK1a* to normal levels in trisomic mice (Ts65Dn) by shRNA also rescues functional and behavioral defects in these mice consistent with a role for increased *DYRK1A* in their pathogenesis. These data are supported by Hibaoui *et al* [152] who reported that impaired neural differentiation of trisomy 21 iPSC was rescued by a selective *DYRK1A* inhibitor. The exact mechanism(s) by which *DYRK1A* affects neurogenesis and neuronal differentiation is not yet clear but includes *DYRK1A*-mediated de-regulation of the master regulator of neuronal differentiation NRSF/REST [153] and inhibition of choline acetyltransferase induction [154].

#### Cardiac abnormalities

Both Ts65Dn and Tc1 mice (Table 2) exhibit heart defects similar to those observed in DS, suggesting that trisomy of one or more of the ~100 genes common to these models may be responsible for the cardiac defects in DS [30,155,156]. Indeed, a recent study

has reported that the smallest critical region in a mouse model (Dp(16)4Yeh/+) associated with cardiac defects, including atrial and ventricular septal defects, can be reduced to a 3.7-Mb region containing 35 genes [157]. Although elegant experiments in transgenic mice provide a guide to the regions of Hsa21 likely to be critical for cardiac defects in individuals with DS, the frequency of these defects in DS mouse models [30,156] is considerably lower (5–15%) than in humans (40–50%; Table 1), suggesting a very complex etiology involving multiple Hsa21 and non-Hsa21 genes.

In comparison with studies in hematopoietic, craniofacial and brain tissue, little is known about the cellular and molecular basis for these defects in cardiac development. Using BrdU labeling, Fuchs *et al* [158] found that neonatal Ts65Dn mice had fewer proliferating cells in the left and right heart walls and septum compared to euploid mice. Interestingly, in the same experiments, they also found reduced numbers of BrdU-positive cells in the intestine, liver and skin in Ts65Dn mice compared to euploid mice, supporting the contention that trisomy 21 impairs the proliferation of progenitor cells of a wide variety of non-hematopoietic tissues during development. The mechanism(s) is not yet clear. Limited gene expression studies of whole human fetal cardiac tissue suggest that dose-dependent upregulation of Hsa21 genes might alter the expression of mitochondrial function genes although this does not explain why cardiac defects affect only half of DS individuals [159]. However, progress in understanding the genetic basis of congenital heart defects, such as AVSD, in the absence of trisomy 21, provides some clues to the potential pathways in DS, including *HOX* genes [160,161], the Shh pathway [162,163], the VEGF pathway [164] and a number of chromatin remodeling genes, including *MLL2* and *CHD7* [reviewed in 165]. Interestingly, Ackerman *et al* [166] recently used a candidate gene resequencing approach to identify potentially damaging variants in six genes at approximately sevenfold higher frequency in DS individuals with AVSD, including 2 Hsa21 genes (*COL6A1*, *COL6A2*) and one gene involved in Wnt signaling (*FRZB*); all six genes identified in this study are implicated in VEGF-A signaling known to be important for normal heart septation [164]. The recent refinements in techniques to generate cardiomyocytes and cardiac progenitors *in vitro* from ESC and iPSC [167] have provided insight into developmental heart defects [168] and may also prove a useful approach for further investigating the mechanisms of defects in cardiac development in DS at the cellular and molecular level [169].

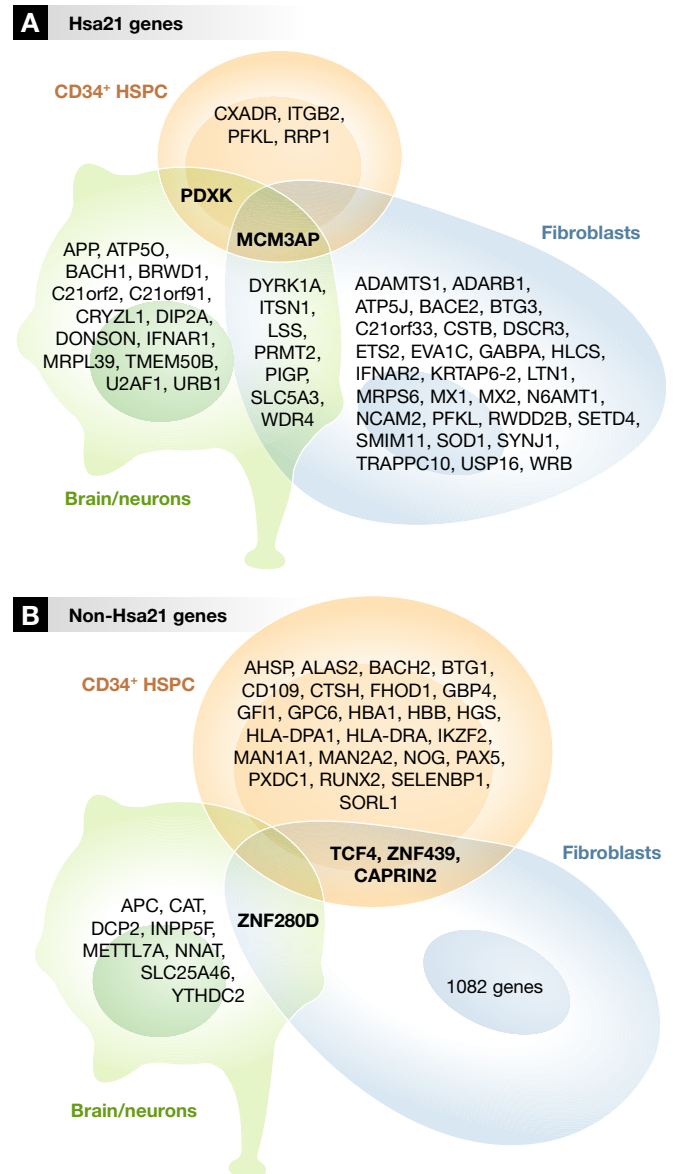
#### Genomic basis for phenotypic variation in DS

Current estimates identify 243 protein-coding genes on Hsa21 as well as 259 long non-coding RNAs and 138 short non-coding RNAs [5]. There is general consensus that changes in the pattern and level of expression of one or more Hsa21 genes are responsible, directly or indirectly, for the abnormalities in stem/progenitor cell function and, ultimately, for the clinical features of DS. The conventional view is that most of these features occur due to imbalanced dosage of Hsa21 genes. However, increasing evidence indicates that the genetic landscape is far more complex than can be accounted for by a simple dosage effect and that trisomy 21 exerts its effects in stem and progenitor cells in several ways.

First, for some Hsa21 genes (probably a minority), mouse models show that in some tissues, increased expression of a single gene is sufficient to produce changes in stem/progenitor cell behavior which correlate with clinical phenotypic read outs, such as leukemia (*ERG*, *DYRK1A*, *HMGN1* or *miR125b*) or cognitive defects (*APP*) [51,91,96–98,118,129]. Conclusive evidence for a simple dosage effect of individual Hsa21 genes in human stem/progenitor cells is lacking at present. A more likely scenario is that several Hsa21 genes, either acting in a common pathway (e.g., NFAT or Wnt signaling) or independently, cause the phenotypic effects in DS [94,95,170]. Interestingly, Emmrich *et al* [95] demonstrated one mechanism by which increased expression of a group of Hsa21 genes might affect several target genes and alter HSPC behavior. They showed that coordinated expression of 3 Hsa21 miRs as a miR99a/let7c/miR125b tricistron by lentiviral transduction of cord blood CD34<sup>+</sup> HSPC caused expansion of megakaryocyte progenitors and modulation of target genes in the TGF $\beta$  and Wnt signaling pathways.

In human tissues, microarray has been used to identify differential expression patterns in trisomic versus euploid cells. Interestingly, in almost all gene expression datasets, only a small minority of the 243 Hsa21 protein-coding genes are significantly differentially expressed (Fig 3; Supplementary Tables S1 and S2). The low number of differentially expressed Hsa21 genes may be due to limited sensitivity of microarray to detect the small changes expression (1.2- to 1.8-fold) expected in trisomic cells [discussed in 92]. Such changes might also be masked by inter-individual variation in expression of Hsa21 genes, as seen for non-Hsa21 genes [93]. Nevertheless, in principle, small changes in expression of multiple Hsa21 genes, each of which is not identified as statistically significant, may still cause critical dysregulation of stem/progenitor cell function. Figure 4 illustrates simple scenarios by which trisomy 21 may lead to a range of possible effects on gene expression and protein production (even without taking into account the impact of epigenetic mechanisms and interindividual variation). Mathematical modeling, supported by experimental evidence [171,172], shows that three dosage effects on gene expression are commonly found in aneuploids: a direct transacting effect, an inverse transacting effect and gene dosage compensation [171,173]. While the direct effect would result in a 1.5-fold increase in expression of genes on the trisomic chromosome, inverse transacting effects (where a gene on the trisomic chromosome regulates a gene on another chromosome) account for otherwise unexpected reductions in gene expression below the normal euploid level. On the other hand, dosage compensation arises when direct and inverse effects are counter-balanced, for example, if a gene on the trisomic chromosome regulates another gene on the same chromosome. Thus, dosage compensation ('buffering') may explain, at least in part, the small number of significantly differentially expressed genes in mouse and human trisomy 21 datasets.

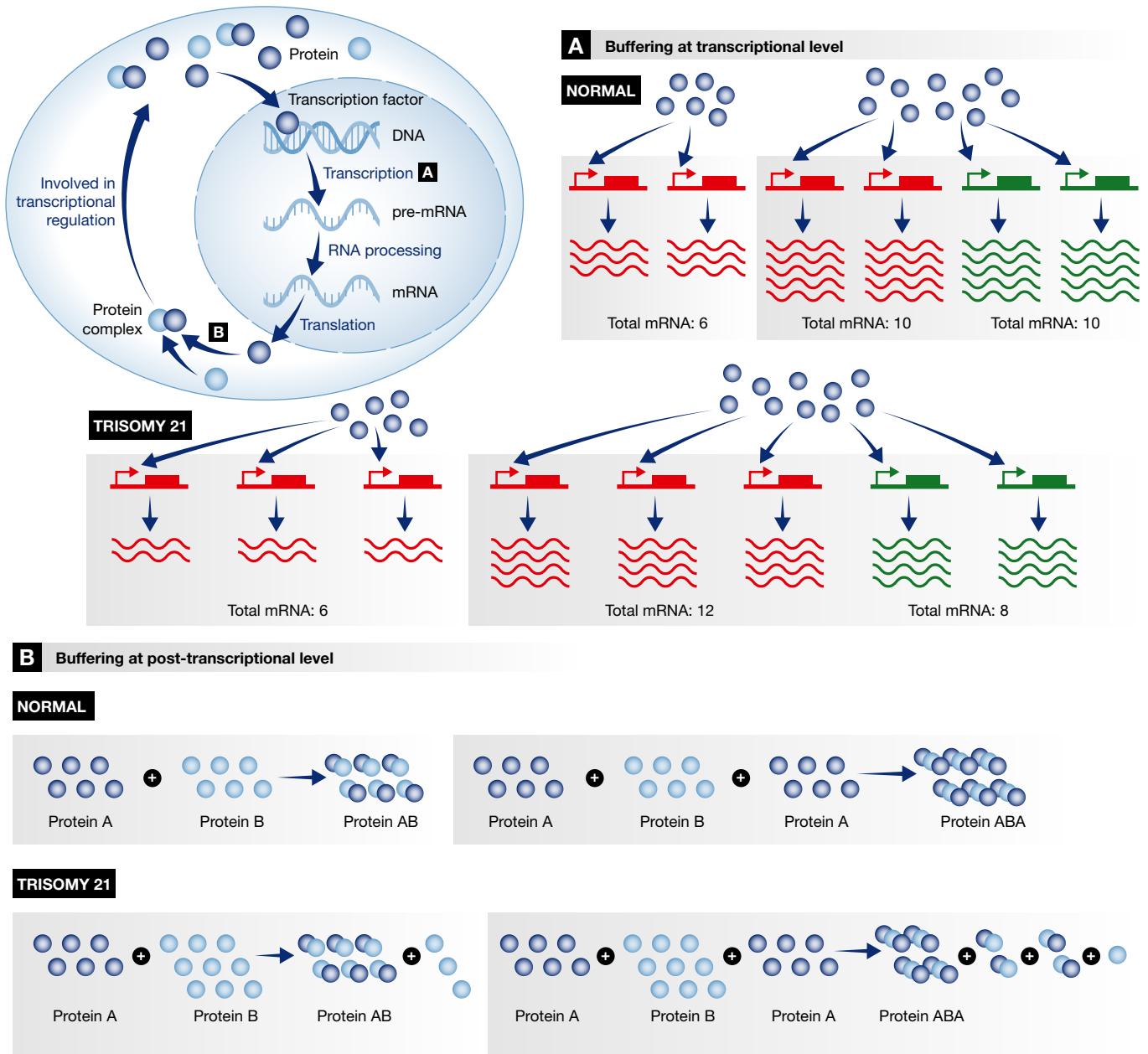
Second, gene expression studies in human DS tissues (Supplementary Tables S1 and S2) [61,71,72,90,93,95,100,102,152,159,174–191], as well as DS mouse models, show extensive dysregulation of non-trisomic (euploid) genes as well as trisomic genes in DS (Fig 3), which are linked to DS-specific phenotypes in DS mouse models [35,93,192]. In a recent fascinating study in fibroblasts and iPSC from a unique set of monozygotic twins where one twin had trisomy 21 and the other did not (as a result of abnormal chromosome segregation prior to twinning), Letourneau *et al* [93] reported



**Figure 3. Differential expression of Hsa21 genes and non-Hsa21 genes in hematopoietic and non-hematopoietic cells.**

Human Gene Expression Profiling (GEP) datasets from public repositories were extracted, and differential expression analysis was performed using the limma algorithm (see Supplementary Tables S1 and S2). The data analyzed here are from references [187,189,190]. In order to compare gene expression by these non-hematopoietic cells with non-malignant hematopoietic cells, we used our own unpublished gene expression data from human trisomy 21 ( $n = 4$ ) and normal ( $n = 3$ ) second-trimester fetal liver CD34<sup>+</sup> HSPC samples obtained using the Affymetrix HuEX\_1-0\_st array. An FDR cutoff of 0.15 was used. There was almost no overlap between the differentially expressed genes in different tissue types: Only a single Hsa21 gene, *MCM3AP* (minichromosome maintenance complex component 3-associated protein) (A), and no non-Hsa21 genes were differentially expressed in both hematopoietic and non-hematopoietic cells (B).

changes in gene expression across every chromosome. Furthermore, they found a consistent pattern of alternating regions of increased and decreased gene expression across large chromosomal segments which they called 'gene expression dysregulation domains' (GEDDs). Remarkably, GEDDs with increased expression in trisomic cells



**Figure 4. Models predicting the consequence of increased gene dosage due to trisomy in stem and progenitor cells.**

This shows various scenarios by which increasing gene dosage to 150% (as in trisomy) may cause a range of effects at the transcriptional and post-transcriptional level which lead to stoichiometric imbalances which may 'buffer' the effect of dosage imbalance of trisomic genes. Trisomy 21 is used as an example. For simplicity, these models do not take into account the additional impact of epigenetic mechanisms and interindividual variation. (A) Effects at the transcriptional level. Left: A gene on Hsa21 (red) is regulated by a transcription factor (blue circle) present in a limited and similar amount in normal and trisomy 21 cells. In this case, transcription is the limiting factor: The additional gene will have no impact on the amount of mRNA produced, and the overall level of expression of the gene is the same in both trisomy 21 and normal (euploid) cells. Right: A gene on Hsa21 (red) is co-regulated by a gene on another chromosome (green) by a transcription factor (blue circle) which is present in limited amounts. The total quantity of mRNA produced is still constrained by the limited quantity of transcription factor which must now be shared between expression of five genes rather than four. The expression of the three copies of the (red) Hsa21 gene will now consume 3/5 of the transcription factor (compared to 2/4 in the euploid state); therefore a scaling of  $(3/5) / (2/4) = 3 \times 4 / (2 \times 5) = 12/10 = 120\%$ . The expression of the two copies of the (green) non-Hsa21 gene will, instead, consume 2/5 instead of 2/4; therefore a scaling of  $(2/5) / (2/4) = 4 \times 2 / (5 \times 2) = 80\%$ . Thus, in this case, the additional copy of Hsa21 causes only a small increase in expression of the Hsa21 gene (to 120%), and this is matched by a decrease in expression (to 80%) of the other, non-Hsa21 gene. (B) Effects at the post-transcriptional level. Buffering effects at the protein level are related to the formation of a complex of proteins. Two examples are illustrated here. Left: A complex AB is formed by a protein A (dark blue circle) and a protein B (light blue circle). If the amount of protein B is increased by a factor of 1.5, but the amount of protein A remains constant, the number of AB complexes will not be increased above the normal level. Right: A complex ABA is formed by a ratio of 2 monomers of protein A and one monomer of protein B through intermediate complexes (AB or BA). When the amount of protein A is exactly twice the amount of protein B, all the proteins are used to form the complexes ABA. However, a 1.5-fold increase in the amount of protein B may lead to a decrease of the amount of ABA complexes since the production of the intermediate AB and BA complexes cannot be completed due to an insufficient amount of A monomers. In addition, as illustrated in the top left of the figure, buffering effects at the protein level may also influence the level of gene expression if the protein complex is itself involved in transcriptional regulation.

corresponded to areas which were normally repressed, while GEDDs with decreased expression corresponded to areas where transcription would normally be active. The mechanism(s) by which the chromatin environment is altered in this way by trisomy 21 has not yet been identified. No comparable data exist for other human trisomies, and so, it is also possible that these effects are due to the physical presence of an additional chromosome in the nucleus rather than specific to Hsa21. It also remains to be seen whether such altered global gene expression patterns are also seen in stem and progenitor cells from other tissues (e.g., HSPC) in individuals with DS.

Since the effects of trisomy 21 on stem/progenitor cells vary depending on the cellular context and stage of development, it is likely that the gene expression patterns responsible for these effects will also vary in different cell types. To address whether there was any overlap in differentially expressed genes between different tissue types in DS, we performed differential expression analysis of non-hematopoietic microarray datasets (Supplementary Tables S1 and S2) with our own gene expression dataset of primary fetal liver CD34<sup>+</sup> HSPC. We found almost no overlap between the differentially expressed genes in different tissue types (Fig 3). Indeed, no non-Hsa21 genes and only a single Hsa21 gene, *MCM3AP*, were differentially expressed in both hematopoietic and non-hematopoietic cells. Although *MCM3AP* may be of interest since it is essential for initiation of DNA replication and mutations in families with inherited intellectual disability have been reported [193], the impact of increased levels of expression on hematopoietic and non-hematopoietic cells is unknown.

## Conclusion

There is increasing recognition that trisomy 21 impacts on stem and progenitor cell function in many different ways. These changes in stem/progenitor cell behavior reflect adaptive epigenetic, transcriptional and/or translational regulatory mechanisms which allow cells to survive and function despite the presence of an additional copy of an entire chromosome. The effects of trisomy 21 on stem and progenitor cells are cell context dependent and developmental stage specific (Fig 1). During fetal and embryonic life, proliferation of hematopoietic and testicular stem and progenitor cells is increased and coupled with altered differentiation, which may underlie the unique susceptibility of individuals with DS to tumors of these two cell types. By contrast, the effects of trisomy 21 on progenitor cells of other lineages (e.g., cardiac, neural and intestinal) during early development are manifested mainly as impaired, rather than enhanced, cell proliferation which may protect these cells from subsequent malignant transformation and explain the reduced frequency of non-hematopoietic cancers in DS. Finally, trisomy 21-mediated premature aging of stem/progenitor cells may contribute to the phenotypic abnormalities in many tissue types, particularly in adults with DS. Although changes in the pattern and level of expression of one or more of these genes on Hsa21 are likely to be responsible, for the abnormalities in stem/progenitor cell function and, ultimately, for the clinical features of DS, increasing evidence indicates that these effects are mediated by complex genetic and epigenetic mechanisms beyond increased Hsa21 gene dosage. Uncovering the molecular mechanisms underpinning these defects in stem and progenitor cell function remains an exciting

### Sidebar A: In need of answers

- The molecular mechanism by which gene dosage is regulated in a developmental stage- and tissue-specific context in trisomic stem and progenitor cells
- Reasons for the variable penetrance of abnormal phenotypes seen in individuals with aneuploidy
- The role of non-coding RNAs in the abnormalities of hematopoietic and non-hematopoietic stem and progenitor cells in human trisomies
- Mechanisms by which epigenetic changes are driven by trisomic genes; either directly or via their effects on other (disomic) genes

challenge and is at last beginning to offer real prospects of translation of these findings into useful therapeutic advances for individuals with DS.

**Supplementary information** for this article is available online: <http://embor.embopress.org>

## Acknowledgements

The authors would like to thank members of the Roberts and Karadimitris labs (especially Tassos Karadimitris, Centre for Haematology, Imperial College London, UK), Professor Michael Stumpf (Centre for Bioinformatics, Division of Molecular Biosciences, Imperial College London, UK) and Professor Paresch Vyas (University of Oxford, UK) and members of his laboratory (especially Kelly Perkins) for helpful discussions over many years. We would also like to thank Professor Vaskar Saha and his laboratory (University of Manchester, UK) and Dr. Ollie Tunstall for help with the microarray experiments in human fetal CD34<sup>+</sup> cells. This work was supported by Leukaemia Lymphoma Research, Children with Cancer and the Medical Research Council.

## Author contributions

BL, SF, AR and IR analyzed data and wrote the paper.

## Conflict of interest

The authors declare that they have no conflict of interest.

## References

1. Morris JK, Springett A (2014) *The National Down Syndrome Cytogenetic Register for England and Wales: 2012 Annual Report*. London: Public Health England
2. Rasmussen SA, Wong LC, May KM, Friedman JM (2003) Population-based analyses of mortality in trisomy 13 and trisomy 18. *Pediatrics* 111: 777–784
3. Nelson KE, Hexem KR, Feudtner C (2012) Inpatient hospital care of children with trisomy 13 and trisomy 18 in the United States. *Pediatrics* 129: 869–876
4. Liang CA, Braddock BA, Heithaus JL, Christensen KM, Braddock SR, Carey JC (2013) Reported communication ability of persons with trisomy 18 and trisomy 13. *Dev Neurorehabil* doi: 10.3109/17518423.2013.847980
5. Wu J, Morris JK (2013) The population prevalence of Down's syndrome in England and Wales in 2011. *Eur J Hum Genet* 21: 1016–1019
6. Antonarakis SE (1998) 10 years of Genomics, chromosome 21, and Down syndrome. *Genomics* 51: 1–16

7. Ross JA, Spector LG, Robison LL, Olshan AF (2005) Epidemiology of leukemia in children with Down syndrome. *Pediatr Blood Cancer* 44: 8–12
8. Antonarakis SE, Lyle R, Dermitzakis ET, Reymond A, Deutsch S (2004) Chromosome 21 and down syndrome: from genomics to pathophysiology. *Nat Rev Genet* 5: 725–738
9. Roper RJ, Reeves RH (2006) Understanding the basis for Down syndrome phenotypes. *PLoS Genet* 2: e50
10. Bull MJ, the Committee on Genetics (2011) Health supervision for children with Down syndrome. *Pediatrics* 128: 393–406
11. Roberts I, Alford K, Hall G, Juban G, Richmond H, Norton A, Vallance G, Perkins K, Marchi E, McGowan S et al (2013) GATA1-mutant clones are frequent and often unsuspected in babies with Down syndrome: identification of a population at risk of leukemia. *Blood* 122: 3908–3917
12. Zigman WB, Schupf N, Sersen E, Silverman W (1996) Prevalence of dementia in adults with and without Down syndrome. *Am J Ment Retard* 100: 403–412
13. Sekijima Y, Ikeda S, Tokuda T, Satoh S, Hidaka H, Hidaka E, Ishikawa M, Yanagisawa N (1998) Prevalence of dementia of Alzheimer type and apolipoprotein E phenotypes in aged patients with Down's syndrome. *Eur Neurol* 39: 234–237
14. Holland AJ, Hon J, Huppert FA, Stevens F (2000) Incidence and course of dementia in people with Down's syndrome: findings from a population-based study. *J Intellect Disabil Res* 44: 138–146
15. Tyrrell J, Cosgrave M, McCarron M, McPherson J, Calvert J, Kelly A, McLaughlin M, Gill M, Lawlor BA (2001) Dementia in people with Down's syndrome. *Int J Geriatr Psychiatry* 16: 1168–1174
16. Coppus A, Evenhuis H, Verberne GJ, Visser F, van Gool P, Eikelenboom P, van Duijn C (2006) Dementia and mortality in persons with Down's syndrome. *J Intellect Disabil Res* 50: 768–777
17. Lott IT (2012) Neurological phenotypes for Down syndrome across the life span. *Prog Brain Res* 197: 101–121
18. Glasson EJ, Dye DE, Bittles AH (2014) The triple challenges associated with age-related comorbidities in Down syndrome. *J Intellect Disabil Res* 58: 393–398
19. Hartley SL, Handen BL, Devenney DA, Hardison R, Mihaila I, Proce JC, Cohen AD, Klunk WE, Mailick MR, Johnson SC et al (2014) Cognitive functioning in relation to brain amyloid- $\beta$  in healthy adults with Down syndrome. *Brain* 137: 2556–2563
20. Adorno M, Sikandar S, Mitra SS, Kuo A, Nicolis Di Robilant B, Haro-Acosta V, Ouadah Y, Quarta M, Rodriguez J, Qian D et al (2013) Usp16 contributes to somatic stem-cell defects in Down's syndrome. *Nature* 501: 380–384
21. Roberts I, Izraeli S (2014) Haematopoietic development and leukaemia in Down syndrome. *Br J Haematol* 167: 587–599
22. Nizetic D, Groet J (2012) Tumorigenesis in Down's syndrome: big lessons from a small chromosome. *Nat Rev Cancer* 12: 721–732
23. Deitz SL, Roper RJ (2011) Trisomic and allelic differences influence phenotypic variability during development of Down syndrome mice. *Genetics* 189: 1487–1495
24. Ruparelia A, Pearn ML, Mobley WC (2013) Aging and intellectual disability: insights from mouse models of Down syndrome. *Dev Disabil Res Rev* 18: 43–50
25. O'Doherty A, Ruf S, Mulligan C, Hildreth V, Errington ML, Cooke S, Sesay A, Modino S, Vanes L, Hernandez D et al (2005) An aneuploid mouse strain carrying human chromosome 21 with down syndrome phenotypes. *Science* 309: 2033–2037
26. Gribble SM, Wiseman FK, Clayton S, Prigmore E, Langley E, Yang F, Maguire S, Fu B, Rajan D, Sheppard O et al (2013) Massively parallel sequencing reveals the complex structure of an irradiated human chromosome on a mouse background in the Tc1 model of Down syndrome. *PLoS ONE* 8: e60482
27. Li Z, Yu T, Morishima M, Pao A, LaDuca J, Conroy J, Nowak N, Matsui S, Shiraishi I, Yu YE (2007) Duplication of the entire 22.9 Mb human chromosome 21 syntenic region on mouse chromosome 16 causes cardiovascular and gastrointestinal abnormalities. *Hum Mol Genet* 16: 1359–1366
28. Yu T, Li Z, Jia Z, Clapcote SJ, Liu C, Li S, Asrar S, Pao A, Chen R, Fan N et al (2010) A mouse model of Down syndrome trisomic for all human chromosome 21 syntenic regions. *Hum Mol Genet* 19: 2780–2791
29. Yu T, Liu C, Belichenko P, Clapcote SJ, Li S, Pao A, Kleschevnikov A, Bechard AR, Asrar S, Chen R et al (2012) Effects of individual segmental trisomies of human chromosome 21 syntenic regions on hippocampal long-term potentiation and cognitive behaviors in mice. *Brain Res* 1366: 162–171
30. Raveau M, Lignon JM, Naleso V, Duchon A, Groner Y, Sharp AJ, Dembele D, Brault V, Herault Y (2012) The *App-Runx1* region is critical for birth defects and electrocardiographic dysfunctions observed in a Down syndrome mouse model. *PLoS Genet* 8: e1002724
31. Lopes Pereira P, Magnol L, Sahun I, Brault V, Duchon A, Prandini P, Gruart A, Bizot J-C, Chadeffaux-Vekemans B, Deutsch S et al (2009) A new mouse model for the trisomy of the *Abcg1-U2af1* region reveals the complexity of the combinatorial genetic code of down syndrome. *Hum Mol Genet* 18: 4756–4769
32. Sahun I, Marechal D, Pereira PL, Nalesso V, Gruart A, Garcia JMD, Antonarakis SE, Dierssen M, Herault Y (2014) Cognition and hippocampal plasticity in the mouse is altered by monosomy of a genomic region implicated in Down syndrome. *Genetics* 197: 899–912
33. Moore CS, Roper RJ (2007) The power of comparative and developmental studies for mouse models of Down syndrome. *Mamm Genome* 18: 431–443
34. Wiseman FK, Alford KA, Tybulewicz VL, Fisher EM (2009) Down syndrome- recent progress and future prospects. *Hum Mol Genet* 18: R75–R83
35. Lana-Elola E, Watson-Scales SD, Fisher EM, Tybulewicz VL (2011) Down syndrome: searching for the genetic culprits. *Dis Model Mech* 4: 586–595
36. Das I, Reeves RH (2011) The use of mouse models to understand and improve cognitive deficits in Down syndrome. *Dis Model Mech* 4: 596–606
37. Duchon A, Raveau M, Chevalier C, Nalesso V, Sharp AJ, Herault Y (2011) Identification of the translocation breakpoints in the Ts65Dn and Ts1Cje mouse lines: relevance for modeling Down syndrome. *Mamm Genome* 22: 674–684
38. Reinholdt LG, Ding Y, Gilbert GJ, Czechanski A, Solzak JP, Roper RJ, Johnson MT, Donahue LR, Lutz C, Davisson MT (2011) Molecular characterization of the translocation breakpoints in the Down syndrome mouse model Ts65Dn. *Mamm Genome* 22: 685–691
39. Edgin JO, Mason GM, Spanò G, Fernández A, Nadel L (2012) Human and mouse cognitive phenotypes in Down syndrome: implications for assessment. *Prog Brain Res* 197: 123–151
40. Zhang L, Fu D, Belichenko PV, Liu C, Kleschevnikov AM, Pao A, Liang P, Clapcote SJ, Mobley WC, Yu YE (2012) Genetic analysis of Down syndrome facilitated by mouse chromosome engineering. *Bioeng Bugs* 3: 8–12

41. Rueda N, Florez J, Martinez-Cue C (2012) Mouse models of Down syndrome to unravel causes of mental disabilities. *Neural Plast* 2012: 584071
42. Liu C, Morishima M, Jiang X, Yu T, Meng K, Kay D, Pao A, Ye P, Parmacek MS, Yu YE (2014) Engineered chromosome-based genetic mapping establishes a 3.7 Mb critical genomic region for Down syndrome-associated heart defects in mice. *Hum Genet* 133: 743–753
43. Tunstall-Pedoe O, Roy A, Karadimitris A, de la Fuente J, Fisk NM, Bennett P, Norton A, Vyas P, Roberts I (2008) Abnormalities in the myeloid progenitor compartment in Down syndrome fetal liver precede acquisition of GATA1 mutations. *Blood* 112: 4507–4511
44. Chou ST, Opalinska JB, Yao Y, Fernandes MA, Kalota A, Brooks JSJ, Choi JK, Gewirtz AM, Danet-Desnoyers G-A, Nemiroff RL et al (2008) Trisomy 21 enhances human fetal erythro-megakaryocytic development. *Blood* 112: 4503–4506
45. Roy A, Cowan G, Mead AJ, Filippi S, Bohn G, Chaidos A, Tunstall O, Chan JKY, Choolani M, Bennett P et al (2012) Perturbation of fetal liver hematopoietic stem and progenitor cell development by trisomy 21. *Proc Natl Acad Sci USA* 109: 17579–17584
46. MacLean GA, Menne TF, Guo G, Sanchez DJ, Park IH, Daley GQ, Orkin SH (2012) Altered hematopoiesis in trisomy 21 as revealed through in vitro differentiation of isogenic induced pluripotent cells. *Proc Natl Acad Sci USA* 109: 17567–17572
47. Chou ST, Byrka-Bishop M, Tober JM, Yao Y, Vandorn D, Opalinska JB, Mills JA, Choi JK, Speck NA, Gadue P et al (2012) Trisomy 21-associated defects in human primitive hematopoiesis revealed through induced pluripotent stem cells. *Proc Natl Acad Sci USA* 109: 17573–17578
48. Kirsammer G, Jilani S, Liu H, Davis E, Gurbuxani S, Le Beau MM, Crispino JD (2008) Highly penetrant myeloproliferative disease in the Ts65Dn mouse model of Down syndrome. *Blood* 111: 767–775
49. Carmichael CL, Majewski IJ, Alexander WS, Metcalf D, Hilton DJ, Hewitt CA, Scott HS (2009) Hematopoietic defects in the Ts1Cje mouse model of Down syndrome. *Blood* 113: 1929–1937
50. Alford KA, Slender A, Vanes L, Li Z, Fisher EM, Nizetic D, Orkin SH, Roberts I, Tybulewicz VL (2010) Perturbed hematopoiesis in the Tc1 mouse model of Down syndrome. *Blood* 115: 2928–2937
51. Malinge S, Bliss-Moreau M, Kirsammer G, Diebold L, Chlon T, Gurbuxani S, Crispino JD (2012) Increased dosage of the chromosome 21 ortholog Dyrk1a promotes megakaryoblastic leukemia in a murine model of Down syndrome. *J Clin Invest* 122: 948–962
52. De Vita S, Canzonetta C, Mullighan C, Delom F, Groet J, Baldo C, Vanes L, Dagna-Bricarelli F, Hoischen A, Veltman J et al (2010) Trisomic dose of several chromosome 21 genes perturbs haematopoietic stem and progenitor cell differentiation in Down's syndrome. *Oncogene* 29: 6102–6114
53. Hasle H, Clemmensen IH, Mikkelsen M (2000) Risks of leukaemia and solid tumours in individuals with Down's syndrome. *Lancet* 355: 165–169
54. Hasle H (2001) Pattern of malignant disorders in individuals with Down's syndrome. *Lancet Oncol* 2: 429–436
55. Hermon C, Alberman E, Beral V, Swerdlow AJ (2001) Mortality and cancer in persons with Down's syndrome, their parents and siblings. *Ann Hum Genet* 65: 167–176
56. Yang Q, Rasmussen SA, Friedman JM (2002) Mortality associated with Down's syndrome in the USA from 1983 to 1997: a population-based study. *Lancet* 359: 1019–1025
57. Hill DA, Gridely G, Cnattingius S, Mellekjær L, Linet M, Adami HQ, Olsen JH, Nyren O, Fraumeni JF Jr (2003) Mortality and cancer incidence among individuals with Down syndrome. *Arch Intern Med* 163: 705–711
58. Goldacre M, Wotton CJ, Seagroate V, Yeates D (2004) Cancers and immune related diseases associated with Down's syndrome: a record linkage study. *Arch Dis Child* 89: 1014–1017
59. Patja K, Pukkala E, Sund R, Iivanainen M, Kaski M (2006) Cancer incidence of persons with down syndrome in Finland: a population-based study. *Int J Cancer* 118: 1769–1772
60. Gilbert D, Rapley E, Shipley J (2011) Testicular germ cell tumours: predisposition genes and the male germ cell niche. *Nat Rev Cancer* 11: 278–288
61. Malinge S, Izraeli S, Crispino JD (2009) Insights into the manifestations, outcomes, and mechanisms of leukemogenesis in Down syndrome. *Blood* 113: 2619–2628
62. Roy A, Roberts I, Vyas P (2012b) Biology and management of transient abnormal myelopoiesis (TAM) in children with Down syndrome. *Semin Fetal Neonatal Med* 17: 196–201
63. Wechsler J, Greene M, McDevitt MA, Anastasi J, Karp JE, Le Beau MM, Crispino JD (2002) Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. *Nat Genet* 32: 148–152
64. Rainis L, Bercovich D, Strehl S, Teigler-Schlegel A, Stark B, Trka J, Amariglio N, Biondi A, Muler I, Rechavi G et al (2003) Mutations in exon 2 of GATA1 are early events in megakaryocytic malignancies associated with trisomy 21. *Blood* 102: 981–986
65. Hitzler JK, Cheung J, Li Y, Scherer SW, Zipursky A (2003) GATA1 mutations in transient leukemia and acute megakaryoblastic leukemia of Down syndrome. *Blood* 101: 4301–4304
66. Groet J, McElwaine S, Spinelli M, Rinaldi A, Burtcher I, Mulligan C, Mensah A, Cavani S, Dagna-Bricarelli F, Basso G et al (2003) Acquired mutations in GATA1 in neonates with Down's syndrome with transient myeloid disorder. *Lancet* 361: 1617–1620
67. Ahmed M, Sternberg A, Hall G, Thomas A, Smith O, O'Marcaigh A, Wynn R, Stevens R, Addison M, King D et al (2004) Natural history of GATA1 mutations in Down syndrome. *Blood* 103: 2480–2489
68. Hollanda LM, Lima CSP, Cunha AF, Albuquerque DM, Vassallo J, Ozelo MC, Joazeiro PP, Saad STO, Costa FF (2006) An inherited mutation leading to production of only the short isoform of GATA-1 is associated with impaired erythropoiesis. *Nat Genet* 38: 807–812
69. Buitenkamp TD, Izraeli S, Zimmermann M, Forestier E, Heerema NA, van den Heuvel-Eibrink MM, Pieters R, Korbijn CM, Silverman LB, Schmiegelow K et al (2014) Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group. *Blood* 123: 70–77
70. Bercovich D, Ganmore I, Scott LM, Wainreb G, Birger Y, Elimelech A, Shochat C, Cazzaniga G, Biondi A, Basso G et al (2008) Mutations of JAK2 in acute lymphoblastic leukaemias associated with Down's syndrome. *Lancet* 372: 1484–1492
71. Hertzberg L, Vendramini E, Ganmore I, Cazzaniga G, Schmitz M, Chalker J, Shiloh R, Iacobucci I, Shochat C, Zeligson S et al (2010) Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the International BFM Study Group. *Blood* 115: 1006–1017
72. Mullighan CG, Collins-Underwood JR, Phillips LA, Loudin MG, Liu W, Zhang J, Ma J, Coustan-Smith E, Harvey RC, Willman CL et al (2009) Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. *Nat Genet* 41: 1243–1246

73. Russell LJ, Capasso M, Vater I, Akasaka T, Bernard OA, Calasanz MJ, Chandrasekaran T, Chapiro E, Gesk S, Griffiths M et al (2009) Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. *Blood* 114: 2688–2698
74. Shochat C, Tal N, Bandapalli OR, Palmi C, Ganmore I, te Kronnie G, Cario G, Cazzaniga G, Kulozik AE, Stanulla M et al (2011) Gain-of-function mutations in interleukin-7 receptor- $\alpha$  (IL7R) in childhood acute lymphoblastic leukemias. *J Exp Med* 208: 901–908
75. Tal N, Shochat C, Geron I, Bercovich D, Izraeli S (2014) Interleukin 7 and thymic stromal lymphopoietin: from immunity to leukemia. *Cell Mol Life Sci* 71: 365–378
76. Nikolaev SI, Garieri M, Santoni F, Falconnet E, Ribaux P, Guipponi M, Murray A, Groet J, Giarin E, Basso G et al (2014) Frequent cases of Ras-mutated Down syndrome acute lymphoblastic leukaemia lack JAK2 mutations. *Nat Commun* 5: 4654–4659
77. Prasher VP (1994) Screening of medical problems in adults with Down syndrome. *Downs Syndr Res Pract* 2: 59–66
78. Zana M, Szecsenyi A, Czibula A, Bjelik A, Juhasz A, Rimanoczy A, Szabo K, Vetro A, Szucs P, Varkonyi A et al (2006) Age-dependent oxidative stress-induced DNA damage in Down's lymphocytes. *Biochem Biophys Res Commun* 345: 726–733
79. Pellegrini FP, Marinoni M, Frangione V, Tedeschi A, Gandini V, Ciglia F, Mortara L, Accolla RS, Nespoli L (2012) Down syndrome, autoimmunity and T regulatory cells. *Clin Exp Immunol* 169: 238–243
80. McLean S, McHale C, Enright H (2009) Hematological abnormalities in adult patients with Down's syndrome. *Ir J Med Sci* 178: 35–38
81. Zipursky A (2000) Susceptibility to leukemia and resistance to solid tumors in Down syndrome. *Pediatr Res* 47: 704
82. Whitlock JA, Sather HN, Gaynon P, Robison LL, Wells RJ, Trigg M, Heerema NA, Bhatia S (2005) Clinical characteristics and outcome of children with Down syndrome and acute lymphoblastic leukemia: a Children's Cancer Group study. *Blood* 106: 4043–4049
83. Kusters MA, Versteegen RH, Gemen EF, de Vries E (2009) Intrinsic defect of the immune system in children with Down syndrome: a review. *Clin Exp Immunol* 156: 189–193
84. Walkley CR, Olsen GH, Dworkin S, Fabb SA, Swann J, McArthur GA, Westmoreland SV, Chambon P, Scadden DT, Purton LE (2007a) A Microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor  $\gamma$  deficiency. *Cell* 129: 1097–1110
85. Walkley CR, Shea JM, Sims NA, Purton LE, Orkin SH (2007b) Rb regulates interactions between hematopoietic stem cells and their bone marrow microenvironment. *Cell* 129: 1081–1095
86. Krause DS, Fulzele K, Catic A, Sun CC, Dombkowski D, Hurley MP, Lezeau S, Attar E, Wu JY, Lin HY et al (2013) Differential regulation of myeloid leukemias by the bone marrow microenvironment. *Nat Med* 19: 1513–1517
87. Kode A, Manavalan JS, Mosialou I, Bhagat G, Rathinam CV, Luo N, Khiabani H, Lee A, Murty VV, Friedman R et al (2014) Leukemogenesis induced by an activating  $\beta$ -catenin mutation in osteoblasts. *Nature* 506: 240–244
88. Klusmann J-H, Creutzig U, Zimmermann M, Dworzak M, Jorch N, Langebrake C, Pekrun A, Macakova-Reinhardt K, Reinhardt D (2008) Treatment and prognostic impact of transient leukemia in neonates with Down syndrome. *Blood* 111: 2991–2998
89. Gamis AS, Woods WG, Alonzo TA, Buxton A, Lange B, Barnard DR, Gold S, Smith FO, Children's Cancer Group Study 2891 (2003) Increased age at diagnosis has a significantly negative effect on outcome in children with Down syndrome and acute myeloid leukemia: a report from the Children's Cancer Group Study 2891. *J Clin Oncol* 21: 3415–3422
90. Klusmann J-H, Godinho FJ, Heitmann K, Maroz A, Koch ML, Reinhardt D, Orkin SH, Li Z (2010) Developmental stage-specific interplay of GATA1 and IGF signaling in fetal megakaryopoiesis and leukemogenesis. *Genes Dev* 24: 1659–1672
91. Ng AP, Hyland CD, Metcalf D, Carmichael CL, Loughran SJ, Di Rago L, Kile BT, Alexander WS (2010) Trisomy of Erg is required for myeloproliferation in a mouse model of Down syndrome. *Blood* 115: 3966–3969
92. Birchler JA (2010) Reflections on studies of gene expression in aneuploids. *Biochem J* 426: 119–123
93. Letourneau A, Santoni FA, Bonilla X, Sailani MR, Gonzalez D, Kind J, Chevalier C, Thurman R, Sandstrom RS, Hibaoui Y et al (2014) Domains of genome-wide gene expression dysregulation in Down's syndrome. *Nature* 508: 345–350
94. Arron JR, Winslow MM, Polleri A, Chang CP, Wu H, Gao X, Neilson JR, Chen L, Heit JJ, Kim SK et al (2006) NFAT dysregulation by increased dosage of DSCR1 and DYRK1A on chromosome 21. *Nature* 441: 595–600
95. Emmrich S, Rasche M, Schoening J, Reimer C, Keihani S, Maroz A, Xie Y, Li Z, Schambach A, Reinhardt D et al (2014) miR99a/100–125b tricistrons regulate hematopoietic stem and progenitor cell homeostasis by shifting the balance between TGF $\beta$  and Wnt signalling. *Genes Dev* 28: 858–874
96. Klusmann JH, Li Z, Bohmer K, Maroz A, Koch ML, Emmrich S, Godinho FJ, Orkin SH, Reinhardt D (2010b) miR-125b-2 is a potential oncomiR on human chromosome 21 in megakaryoblastic leukemia. *Genes Dev* 24: 478–490
97. Birger Y, Goldber L, Chlon TM, Goldenson B, Muler I, Schiby G, Jacob-Hirsch J, Rechavi G, Crispino JD, Izraeli S (2013) Perturbation of fetal hematopoiesis in a mouse model of Down syndrome's transient myeloproliferative disorder. *Blood* 122: 988–998
98. Lane AA, Chapuy B, Lin CY, Tivey T, Li H, Townsend EC, van Bodegom D, Day TA, Wu SC, Liu H et al (2014) Triplication of a 21q22 region contributes to B cell transformation through HMGN1 overexpression and loss of histone H3 Lys27 trimethylation. *Nat Genet* 46: 618–623
99. Salek-Ardakani S, Smootha G, de Boer J, Sebire NJ, Morrow M, Rainis L, Lee S, Williams O, Izraeli S, Brady HJ (2009) ERG is a megakaryocytic oncogene. *Cancer Res* 69: 4665–4673
100. Bourquin JP, Subramanian A, Langebrake C, Reinhardt D, Bernard O, Ballerini P, Baruchel A, Cave H, Dastugue N, Hasle H et al (2006) Identification of distinct molecular phenotypes in acute megakaryoblastic leukemia by gene expression profiling. *Proc Natl Acad Sci USA* 103: 3339–3344
101. Hussein K, Bock O, Theophile K, Schultz-Bischof K, Porwit A, Schlue J, Jonigk D, Kreipe H (2009) MPLW515L mutation in acute megakaryoblastic leukaemia. *Leukemia* 23: 852–855
102. Yoshida K, Toki T, Okuno Y, Kanezaki R, Shiraishi Y, Sato-Otsubo A, Sanada M, Park MJ, Terui K, Suzuki H et al (2013) The landscape of somatic mutations in Down syndrome-related myeloid disorders. *Nat Genet* 45: 1293–1299
103. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, Cuker A, Wernig G, Moore S, Galinsky I et al (2006) MPLW515L is a novel somatic mutation in myelofibrosis with myeloid metaplasia. *PLoS Med* 3: e270
104. Carmeliet G, David G, Cassiman JJ (1991) Cellular ageing of Alzheimer's disease and Down syndrome cells in culture. *Mutat Res* 256: 221–231

105. Kimura M, Cao X, Skurnick J, Cody M, Soteropoulos P, Aviv A (2005) Proliferation dynamics in cultured skin fibroblasts from Down syndrome subjects. *Free Radic Biol Med* 39: 374–380
106. Jacobs JJ, Lieboom K, Marino S, DePinho RA, van Lohuizen M (1999) The oncogene and Polycomb-group gene *bmi* regulates cell proliferation and senescence through the *ink4* locus. *Nature* 397: 164–168
107. Collado M, Blasco MA, Serrano M (2007) Cellular senescence in cancer and aging. *Cell* 130: 223–233
108. Gil J, O’Loughlin A (2014) PRC1 complex diversity: where is it taking us? *Trends Cell Biol* 24: 632–641
109. Jacobsen GK, Henriques UV (1992) A fetal testis with intratubular germ cell neoplasia (ITGCN). *Mod Pathol* 5: 547–549
110. Satge D, Jacobsen GK, Cessot F, Raffi F, Vekemans M (1996) A fetus with Down syndrome and intratubular germ cell neoplasia. *Pediatr Pathol Lab Med* 16: 107–112
111. Sussan TE, Yang A, Li F, Ostrowski MC, Reeves RH (2008) Trisomy represses *Apc*(Min)-mediated tumours in mouse models of Down’s syndrome. *Nature* 451: 73–75
112. Baek KH, Zaslavsky A, Lynch RC, Britt C, Okada Y, Siarey RJ, Lensch MW, Park IH, Yoon SS, Minami T et al (2009) Down’s syndrome suppression of tumour growth and the role of the calcineurin inhibitor *DSCR1*. *Nature* 459: 1126–1130
113. Yang A, Reeves RH (2011) Increased survival following tumorigenesis in Ts65Dn mice that model Down syndrome. *Cancer Res* 71: 3573–3581
114. Richtsmeier JT, Baxter LL, Reeves RH (2000) Parallels of craniofacial maldevelopment in Down syndrome and Ts65Dn mice. *Dev Dyn* 217: 137–145
115. Richtsmeier JT, Zumwalt A, Carlson EJ, Epstein CJ, Reeves RH (2002) Craniofacial phenotypes in segmentally trisomic mouse models for Down syndrome. *Am J Med Genet* 107: 317–324
116. Trainor PA (2005) Specification and patterning of neural crest cells during craniofacial development. *Brain Behav Evol* 66: 266–280
117. Roper RJ, VanHorn JF, Cain CC, Reeves RH (2009) A neural crest deficit in Down syndrome mice is associated with deficient mitotic response to Sonic hedgehog. *Mech Dev* 126: 212–219
118. Trazzi S, Mitrugno VM, Valli E, Fuchs C, Rizzi S, Guidi S, Perini G, Bartesaghi R, Ciani E (2011) APP-dependent up-regulation of *Ptch1* underlies proliferation impairment of neural precursors in Down syndrome. *Hum Mol Genet* 20: 1560–1573
119. Roper RJ, Baxter LL, Saran NG, Klinedinst DK, Beachy PA, Reeves RH (2006) Defective cerebellar response to mitogenic Hedgehog signaling in Down’s syndrome mice. *Proc Natl Acad Sci USA* 103: 1452–1456
120. Billingsley CN, Allen JR, Baumann DD, Deitz SL, Blazek JD, Newbauer A, Darrah A, Long BC, Young B, Clement M et al (2013) Non-trisomic homeobox gene expression during craniofacial development in the Ts65Dn mouse model of Down syndrome. *Am J Med Genet A* 161: 1866–1874
121. Matsuo I, Kuratani S, Kimura C, Takeda N, Aizawa S (1995) Mouse *Otx2* functions in the formation and patterning of rostral head. *Genes Dev* 9: 2646–2658
122. Degenhardt K, Rentschler S, Fishman G, Sassoon DA (2002) Cellular and cis-regulation of *En-2* expression in the mandibular arch. *Mech Dev* 111: 125–136
123. Sumarsono SH, Wilson TJ, Tymms MJ, Ventner DJ, Corrick CM, Kola R, Lahoud MH, Papas TS, Seth A, Kola I (1996) Down’s syndrome-like skeletal abnormalities in *Ets2* transgenic mice. *Nature* 379: 534–537
124. Hammerle B, Lizalde C, Tejedor FJ (2008) The spatio-temporal and subcellular expression of the candidate Down syndrome gene *Mnb/Dyrk1A* in the developing mouse brain suggests distinct sequential roles in neuronal development. *Eur J Neurosci* 27: 1061–1074
125. Altafaj X, Dierssen M, Baamonde C, Marti E, Visa J, Guimera J, Oset M, Gonzalez JR, Florez J, Fillat C et al (2001) Neurodevelopmental delay, motor abnormalities and cognitive deficits in transgenic mice over-expressing *Dyrk1A* (minibrain), a murine model of Down’s syndrome. *Hum Mol Genet* 10: 1915–1923
126. Olson LE, Richtsmeier J, Leszl J, Reeves RH (2004) A chromosome 21 critical region does not cause specific Down syndrome phenotypes. *Science* 306: 687–690
127. Belichenko PV, Masliah E, Kleschevnikov AM, Villar AJ, Epstein CJ, Salehi A, Mobley WC (2004) Synaptic structural abnormalities in the Ts65Dn mouse model of Down syndrome. *J Comp Neurol* 480: 281–298
128. Hill CA, Sussan TE, Reeves RH, Richtsmeier JT (2009) Complex contributions of *Ets2* to craniofacial and thymus phenotypes of trisomic “Down syndrome” mice. *Am J Med Genet A* 149: 2158–2165
129. Trazzi S, Fuchs C, Valli E, Perini G, Bartesaghi R, Ciani E (2013) The amyloid precursor protein (APP) triplicated gene impairs neuronal precursor differentiation and neurite development through two different domains in the Ts65Dn mouse model for down syndrome. *J Biol Chem* 288: 20817–20829
130. Haydar TF, Reeves RH (2012) Trisomy 21 and early brain development. *Trends Neurosci* 35: 81–91
131. Contestabile A, Fila T, Ceccarelli C, Bonasoni P, Bonapace L, Santini D, Bartesaghi R, Ciani E (2007) cell cycle alteration and decreased cell proliferation in the hippocampal dentate gyrus and in the neocortical germinal matrix of fetuses with Down syndrome and in Ts65Dn mice. *Hippocampus* 17: 665–678
132. Guidi S, Bonasoni P, Ceccarelli C, Santini D, Gualtieri F, Ciani E, Bartesaghi R (2008) Neurogenesis impairment and increased cell death reduce total neuron number in the hippocampal region of fetuses with Down syndrome. *Brain Pathol* 18: 180–197
133. Guidi S, Caina E, Bonasoni P, Santini D, Bartesaghi R (2011) Widespread proliferation impairment and hypocellularity in the cerebellum of fetuses with down syndrome. *Brain Pathol* 21: 361–373
134. Baxter LL, Moran TH, Richtsmeier JT, Troncoso J, Reeves RH (2000) Discovery and genetic localization of Down syndrome cerebellar phenotypes using the Ts65Dn mouse. *Hum Mol Genet* 9: 195–202
135. Lorenzi HA, Reeves RH (2006) Hippocampal hypocellularity in the Ts65Dn mouse originates early in development. *Brain Res* 1104: 153–159
136. Chakrabarti L, Galdzicki Z, Haydar TF (2007) Defects in embryonic neurogenesis and initial synapse formation in the forebrain of the Ts65Dn mouse model of Down syndrome. *J Neurosci* 27: 11483–11495
137. Bianchi P, Ciani E, Guidi S, Trazzi S, Felice D, Grossi G, Fernandez M, Giuliani A, Calza L, Bartesaghi R (2010) Early pharmacotherapy restores neurogenesis and cognitive performance in the Ts65Dn mouse model for Down syndrome. *J Neurosci* 30: 8769–8779
138. Contestabile A, Fila T, Bartesaghi R, Ciani E (2009) Cell cycle elongation impairs proliferation of cerebellar granule cell precursors in the Ts65Dn mouse, an animal model for Down syndrome. *Brain Pathol* 19: 224–237
139. Altafaj X, Martin ED, Ortiz-Abalia J, Valderrama A, Lao-Peregrin C, Dierssen M, Fillat C (2013) Normalization of *Dyrk1a* expression by *AAV2/1-shDyrk1A* attenuates hippocampal-dependent defects in the Ts65Dn mouse model of Down syndrome. *Neurobiol Dis* 52: 117–127



140. Olson LE, Roper RJ, Sengstaken CL, Peterson EA, Aquino V, Galdzicki Z, Siarey R, Pletnikov M, Moran TH, Reeves RH (2007) Trisomy for the Down syndrome 'critical region' is necessary but not sufficient for brain phenotypes of trisomic mice. *Hum Mol Genet* 16: 774–782
141. Das I, Park JM, Shin JH, Jeon SK, Lorenzi H, Linden DJ, Worley PF, Reeves RH (2013) Hedgehog agonist therapy corrects structural and cognitive defects in a Down syndrome mouse model. *Sci Transl Med* 5: 201ra120
142. Guidi S, Stagni F, Bianchi P, Ciani E, Giacomini A, De Franceschi M, Moldrich R, Kurniawan N, Mardon K, Giuliani A et al (2014) Prenatal pharmacotherapy rescues brain development in a Down's syndrome mouse model. *Brain* 137: 380–401
143. Trazzi S, Fuchs C, De Franceschi M, Mitrugno VM, Bartesaghi R, Ciani E (2014) APP-dependent alteration of GSK3 $\beta$  activity impairs neurogenesis in the 65Dn mouse model of Down syndrome. *Neurobiol Dis* 67: 24–36
144. Wechsler-Reya RJ, Scott MP (1999) Control of neuronal precursor proliferation in the cerebellum by Sonic Hedgehog. *Neuron* 22: 103–114
145. Hur EM, Zhou FQ (2010) GSK3 signalling in neural development. *Nat Rev Neurosci* 11: 539–551
146. Clark S, Schwalbe J, Stasko MR, Yarowsky PJ, Costa AC (2006) Fluoxetine rescues deficient neurogenesis in hippocampus of the Ts65Dn mouse model for Down syndrome. *Exp Neurol* 200: 256–261
147. Guedi F, Pereira PL, Najas S, Barallobre MJ, Chabert C, Souchet B, Sebric C, Verney C, Herault Y, Arbones M et al (2012) DYRK1A: a master regulatory protein controlling brain growth. *Neurobiol Dis* 46: 190–203
148. Thomazeau A, Lassalle O, Iafrazi J, Souchet B, Guedi F, Janel J, Chavis P, Delabar J, Manzoni OJ (2014) Prefrontal deficits in a murine model overexpressing the down syndrome candidate gene dyrk1a. *J Neurosci* 34: 1138–1147
149. Souchet B, Guedi F, Sahun I, Duchon A, Daubigny F, Badel A, Yanagawa Y, Barallobre MJ, Dierssen M, Yu E et al (2014) Excitation/inhibition balance and learning are modified by Dyrk1a gene dosage. *Neurobiol Dis* 69: 65–75
150. Garcia-Cerro S, Martinez P, Vidal V, Corrales A, Florez J, Vidal R, Rueda N, Arbones M, Martinez-Cue C (2014) Overexpression of Dyrk1A is implicated in several cognitive, electrophysiological and neuromorphological alterations found in a mouse model of Down syndrome. *PLoS ONE* 9: e106572
151. Ortiz-Abalia J, Sahun I, Altafaj X, Andreu N, Estivill X, Dierssen M, Fillat C (2008) Targeting Dyrk1A with AAVshRNA attenuates motor alterations in TgDyrk1A, a mouse model of Down syndrome. *Am J Hum Genet* 83: 479–488
152. Hibaoui Y, Grad I, Letourneau A, Sailani MR, Dahoun S, Santoni FA, Gimelli S, Guipponi M, Pelte MF, Bene F et al (2013) Modelling and rescuing neurodevelopmental defect of Down syndrome using induced pluripotent stem cells from monozygotic twins discordant for trisomy 21. *PLoS Genet* 9: e1003515
153. Canzonetta C, Mulligan C, Deutsch S, Ruf S, O'Doherty A, Lyle R, Borel C, Lin-Marq N, Delom F, Groet J et al (2008) DYRK1A-dosage imbalance perturbs NRSF/REST levels, deregulating pluripotency and embryonic stem cell fate in Down syndrome. *Am J Hum Genet* 83: 388–400
154. Hijazi M, Fillat C, Medina JM, Velasco A (2013) Overexpression of DYRK1A inhibits choline acetyltransferase induction by oleic acid in cellular models of Down syndrome. *Exp Neurol* 239: 229–234
155. Williams AD, Mjaatvedt CH, Moore CS (2008) Characterization of the cardiac phenotype in neonatal Ts65Dn mice. *Dev Dyn* 237: 426–435
156. Dunlevy L, Bennett M, Slender A, Lana-Elola E, Tybulewicz VL, Fisher EM, Mohun T (2010) Down's syndrome-like cardiac developmental defects in embryos of the transchromosomal Tc1 mouse. *Cardiovasc Res* 88: 287–295
157. Liu C, Morishima M, Jiang X, Yu T, Meng K, Ray D, Pao A, Ye P, Parmacek MS, Yu YE (2014) Engineered chromosome-based genetic mapping establishes a 3.7 Mb critical genomic region for Down syndrome-associated heart defects in mice. *Hum Genet* 133: 743–753
158. Fuchs C, Ciani E, Guidi S, Trazzi S, Bartesaghi R (2012) Early-occurring proliferation defects in peripheral tissues of the Ts65Dn mouse model of Down syndrome are associated with patched1 over expression. *Lab Invest* 92: 1648–1660
159. Conti A, Fabbrini F, D'Agostino P, Negri R, Greco D, Genesio R, D'Armiento M, Olla C, Paladini D, Zannini M et al (2007) Altered expression of mitochondrial and extracellular matrix genes in the heart of human fetuses with chromosome 21 trisomy. *BMC Genom* 8: 268
160. Bertrand N, Roux M, Ryckebusch L, Niederreither K, Dolle P, Moon A, Capecchi M, Zaffran S (2011) Hox genes define distinct progenitor sub-domains within the second heart field. *Dev Biol* 353: 266–274
161. Laforest B, Bertrand N, Zaffran S (2014) Genetic lineage tracing of anterior Hox expressing cells. *Methods Mol Biol* 1196: 37–48
162. Goddeeris MM, Rho S, Petiet A, Davenport CL, Johnson GA, Meyers EN, Klingensmith J (2008) Intracardiac septation requires hedgehog-dependent cellular contributions from outside the heart. *Development* 135: 1887–1895
163. Hoffmann AD, Peterson MA, Friedland-Little JM, Anderson SA, Moskowitz IP (2009) Sonic hedgehog is required in pulmonary endoderm for atrial septation. *Development* 136: 1761–1770
164. Dor Y, Camenisch TD, Itin A, Fishman GI, McDonald JA, Carmeliet P, Kesheti E (2001) A novel role for VEGF in endocardial cushion formation and its potential contribution to congenital heart defects. *Development* 128: 1531–1538
165. Yuan S, Zaidi S, Brueckman M (2013) Congenital heart disease: emerging themes linking genetics and development. *Curr Opin Genet Dev* 23: 352–359
166. Ackerman C, Locke AE, Feingold E, Reshey B, Espana K, Thusberg J, Mooney S, Bean LJ, Dooley KJ, Cua CL et al (2012) An excess of deleterious variants in VEGF-A pathway genes in Down-syndrome-associated atrioventricular septal defects. *Am J Hum Genet* 91: 646–659
167. Matsa E, Burrige PW, Wu JC (2014) Human stem cells for modelling heart disease and for drug discovery. *Sci Trans Med* 6: 239ps
168. Jiang Y, Habibollah S, Tilgner K, Collin J, Barta T, Al-Aama JY, Tesarov L, Hussain R, Trafford AW, Kirkwood G et al (2014) An induced pluripotent stem cell model of hypoplastic left heart syndrome (HLHS) reveals multiple expression and functional defects in HLHS-derived cardiac myocytes. *Stem Cells Trans Med* 3: 416–423
169. Copplola A, Romito A, Borel C, Gehrig C, Gagnebin M, Falconnet E, Izzo A, Altucci L, Banfi S, Antonarakis SE et al (2014) Cardiomyogenesis is controlled by the miR99a/let-7c cluster and epigenetic modification. *Stem Cell Res* 12: 323–337
170. Nikolaev SI, Santoni F, Vannier A, Falconnet E, Giarin E, Basso G, Hoischen A, Veltman JA, Groet J, Nizetic D, et al (2013) Exome sequencing identifies putative drivers of progression of transient

- myeloproliferative disorder to AMKL in infants with Down syndrome. *Blood* 122: 554–561
171. Veitia RA, Bottani S, Birchler JA (2013) Gene dosage effects: nonlinearities, genetic interactions, and dosage compensation. *Trends Genet* 29: 385–393
  172. Sheltzer JM, Torres EM, Dunham MJ, Amon A (2012) Transcriptional consequences of aneuploidy. *Proc Natl Acad Sci USA* 109: 12644–12649
  173. Birchler JA, Veitia RA (2012) Gene balance hypothesis: connecting issues of dosage sensitivity across biological disciplines. *Proc Natl Acad Sci USA* 109: 14746–14753
  174. Jiang J, Jing Y, Cost GJ, Chiang JC, Kolpa HJ, Cotton AM, Carone DM, Carone BR, Shivak DA, Guschin DY et al (2013) Translating dosage compensation to trisomy 21. *Nature* 500: 296–300
  175. Granese B, Scala I, Spatuzza C, Valentino A, Coletta M, Vacca RA, De Luca P, Andria G (2013) Validation of microarray data in human lymphoblasts shows a role of the ubiquitin-proteasome system and NF- $\kappa$ B in the pathogenesis of Down syndrome. *BMC Med Genomics* 6: 24
  176. Li LB, Chang KH, Wang PR, Hirata RK, Papayannopoulou T, Rissell DW (2012) Trisomy correction in Down syndrome induced pluripotent stem cells. *Cell Stem Cell* 11: 615–619
  177. Chou CY, Liu LY, Chen CY, Tsai CH, Hwa HL, Chang LY, Lin YS, Hsieh FJ (2008) Gene expression variation increase in trisomy 21 tissues. *Mamm Genome* 19: 398–405
  178. Slonim DK, Koide K, Johnson KL, Tantravahi U, Cowan JM, Jarrah Z, Bianchi DW (2009) Functional genomic analysis of amniotic fluid cell-free mRNA suggests that oxidative stress is significant in Down syndrome fetuses. *Proc Natl Acad Sci USA* 106: 9425–9429
  179. Volk M, Mayer A, Lovrecic L, Luvan P, Peterlin B (2013) Expression signature as a biomarker for prenatal diagnosis of trisomy 21. *PLoS ONE* 8: e74184
  180. Altug-Teber O, Bonin M, Walter M, Mau-Holzmann UA, Dufke A, Stappert H, Tekesin I, Heilbronner H, Nieselt K, Reiss O (2007) Specific transcriptional changes in human fetuses with autosomal trisomies. *Cytogenet Genome Res* 119: 171–184
  181. Li C, Jin L, Bai Y, Chen Q, Fu L, Yang M, Xiao H, Zhao G, Wang S (2012) Genome-wide expression analysis in Down syndrome: on-site into immunodeficiency. *PLoS ONE* 7: e49130
  182. Loudin MG, Wang J, Leung HC, Gurusiddappa S, Meyer J, Condos G, Morrison D, Tsimelzon A, Devidas M, Heerema NA et al (2011) Genomic profiling in Down syndrome acute lymphoblastic leukemia identifies histone gene deletions associated with altered methylation profiles. *Leukemia* 25: 1555–1563
  183. McElwaine S, Mullighan C, Groet J, Spinelli M, Rinaldi A, Denyer G, Mensah A, Cavani S, Baldo C, Dagna-Bricarelli F et al (2004) Microarray transcript profiling distinguishes the transient from the acute type of megakaryoblastic leukemia (M7) in Down's syndrome, revealing PRAME as a specific discriminating marker. *Br J Haematol* 125: 729–742
  184. Xavier AC, Edwards H, Dombkowski AA, Balci TB, Berman JN, Dellaire G, Xie C, Buck SA, Matherly LH, Ge Y et al (2011) A unique role of Gata1s in Down syndrome acute megakaryocytic leukemia biology and therapy. *PLoS ONE* 6: e27486
  185. Maroz A, Stachorski L, Emmrich S, Reinhardt K, Xu J, Shao Z, Käbler S, Dertmann T, Hitzler J, Roberts I et al (2014) GATA1s induces hyperproliferation of eosinophil precursors in Down syndrome transient leukemia. *Leukemia* 28: 1259–1270
  186. Mao R, Wang X, Spitznagel EL, Frelin LP, Ting JC, Ding H, Kim JW, Ruczinski I, Downey TJ, Pevsner J (2005) Primary and secondary transcriptional effects in the developing human Down syndrome brain and heart. *Genome Biol* 6: R107
  187. Briggs JA, Sun J, Shepherd J, Ovchinnikov DA, Chung TL, Nayler SP, Kao LP, Morrow CA, Thakar NY, Soo SY et al (2013) Integration-free induced pluripotent stem cells model genetic and neural developmental features of down syndrome etiology. *Stem Cells* 31: 467–478
  188. Helguera P, Seiglie J, Rodriguez J, Hanna M, Helguera G, Busciglio J (2013) Adaptive downregulation of mitochondrial function in down syndrome. *Cell Metab* 17: 132–140
  189. Lockstone HE, Harris LW, Swatton JE, Wayland MT, Holland AJ, Bahn S (2007) Gene expression profiling in the adult Down syndrome brain. *Genomics* 90: 647–660
  190. Weick JP, Held DL, Bonadurer GF, Doers ME, Liu Y, Maguire C, Clark A, Knackert JA, Molinarolo K, Musser M et al (2013) Deficits in human trisomy 21 iPSCs and neurons. *Proc Natl Acad Sci USA* 110: 9962–9967
  191. Jin S, Lee YK, Lim YC, Zheng Z, Lin XM, Ng DP, Holbrook JD, Law HY, Kwek KY, Yeo GS et al (2013) Global DNA hypermethylation in down syndrome placenta. *PLoS Genet* 9: e1003515
  192. Laffaire J, Rivals I, Dauphinot L, Pasteau F, Wherle R, Larrat B, Vitalis T, Moldrich RX, Rossier J, Sinkus R et al (2009) Gene expression signature of cerebellar hypoplasia in a mouse model of Down syndrome during postnatal development. *BMC Genom* 10: 138
  193. Schuurs-Hoeijmakers JH, Vulto-van Silfhout AT, Vissers LE, van de Vondervoort II, van Bon BW, de Ligt J, Gilissen C, Hehir-Kwa JY, Neveling K, del Rosario M et al (2013) Identification of pathogenic gene variants in small families with intellectually disabled siblings by exome sequencing. *J Med Genet* 50: 802–811
  194. Guihard-Costa AM, Khung S, Delbecque K, Menez F, Delezoide AL (2006) Biometry of face and brain in fetuses with trisomy 21. *Pediatr Res* 59: 33–38
  195. Allanson JE, O'Hara P, Farkas LG, Nair RC (1993) Anthropometric craniofacial pattern profiles in Down syndrome. *Am J Med Genet* 47: 748–752
  196. Hawli Y, Nasrallah M, El-Hajj Fuleihan G (2009) Endocrine and musculoskeletal abnormalities in patients with Down syndrome. *Nat Rev Endocrinol* 5: 327–334
  197. Patterson T, Rapsey CM, Glue P (2013) Systematic review of cognitive development across childhood in Down syndrome: implications for treatment interventions. *J Intellect Disab Res* 57: 306–318
  198. Margallo-Lana ML, Moore PB, Kay DW, Perry RH, Reid BE, Berney TP, Tyrer SP (2007) Fifteen-year follow up of 92 hospitalized adults with Down's syndrome: incidence of cognitive decline, its relationship to age and neuropathology. *J Intellect Disabil Res* 51: 463–477
  199. Lockrow JP, Fortress AM, Granholm AC (2012) Age-related neurodegeneration and memory loss in down syndrome. *Curr Gerontol Geriatr Res* 2012: 463909
  200. Carroll KN, Arbogast PG, Dudley JA, Cooper WO (2008) Increase in incidence of medically treated thyroid disease in children with Down syndrome after release of American Academy of Pediatrics Health Supervision guidelines. *Pediatrics* 122: e493–e498
  201. Graber E, Chacko E, Regelmann MO, Costin G, Rapaport R (2012) Down syndrome and thyroid function. *Endocrinol Metab Clin North Am* 41: 735–745

202. Freeman SB, Taft LF, Dooley KJ, Allran K, Sherman SL, Hassold TJ, Khoury MJ, Saker DM (1998) Population-based study of congenital heart disease in Down syndrome. *Am J Hum Genet* 80: 213–217
203. Weijerman ME, van Furth AM, van der Mooren MD, van Weissenbruch MM, Rammeloo L, Broers CJ, Gemke RJ (2010) Prevalence of congenital heart disease and persistent pulmonary hypertension of the neonate with Down syndrome. *Eur J Pediatr* 169: 1195–1199
204. Roizen NJ, Magyar CI, Kuschner ES, Sulkes SB, Druschel C, van Wijngaarden E, Rodgers L, Diehl A, Lowry R, Hyman SL (2014) A community cross-sectional survey of medical problems in 440 children with Down syndrome in New York State. *J Pediatr* 164: 871–875