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Influence of allelic differences in Down syndrome

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Abstract

Both trisomic and non-trisomic genes may affect the incidence and severity of phenotypes associated with Down syndrome (DS). The importance of extra (trisomic) genetic material is emphasized in DS, with less emphasis to the allelic composition of candidate trisomic genes in defining the trisomic gene-phenotype relationship in DS. Allelic differences in non-trisomic genes have been shown to be important moderators of cardiac, leukemia, and developmental phenotypes associated with DS. Trisomic mouse models provide an in vivo genetic platform for examining the gene-phenotype relationship, including the influence of allelic variants, on DS-like phenotypes. DS mouse models have differing trisomic genetic makeup, and optimal development, viability and translational value of these mouse models may require a non-inbred genetic background with heterogeneity at many loci. Additionally, understanding the contribution of specific genes or regions to DS phenotypes often requires the utilization of genetically manipulated mice that may be established on a different inbred background than the trisomic mice. The impact of allelic differences of trisomic and background genes in human and model systems may offer insight into the variability in occurrence and severity of trisomic phenotypes.

Keywords

Allelic Variation; Trisomy 21; Down Syndrome; Phenotypic Variability; Mouse Models

Allelic differences are important in the occurrence and severity of traits in individuals with DS

Approximately 95% of individuals with Down syndrome (DS) have full trisomy of human chromosome 21 (Hsa21) (Bull et al., 2011, Van Cleve and Cohen, 2006). Others with DS may have partial trisomy or mosaicism. All individuals with DS carry a subset of ~80 defined traits; including cognitive impairment, distinctive craniofacial structure, and cardiac anomalies (Epstein, 2001, Bull et al., 2011). Individuals with partial trisomy 21 (Ts21) or mosaicism may have reduced overall prevalence and severity of DS phenotypes. Yet even with a full extra copy of Hsa21, there is a high degree of phenotypic variability observed in individuals with Ts21. Genetic analyses suggest larger numbers of trisomic genes may

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produce a more comprehensive DS phenotype, but prevalence or severity of individual traits may not result directly from increased trisomic content (Olson et al., 2004b, Korbel et al., 2009, Lyle et al., 2009). Phenotypic variability may be associated with allelic differences in trisomic or non-trisomic (euploid) genes.

There are a number of premises of how allelic differences [sequence variants or polymorphisms (Richards et al., 2015)] may influence the incidence and severity of phenotypes associated with DS. These include hypotheses about how allelic differences on the trisomic chromosome may affect survival and DS phenotypes, how trisomy may provide a sensitized background for allelic differences throughout the genome and how trisomy may facilitate surpassing a certain genetic threshold to cause a phenotypic deficit. [Sensitization in the context of this article means increased vulnerability to the adverse phenotypes due to trisomy]. Allelic variation or sequence differences may occur in all parts of the genome and may affect exons, introns, promoters, untranslated regions (UTR) or non-coding DNA to alter genes and proteins that are important in a particular phenotype (Riordan and Nadeau, 2017). The frequency of allelic differences in both trisomic and euploid genes may influence phenotypic variability in all traits of individuals with DS (Roper and Reeves, 2006). This chapter examines hypotheses of allelic differences in trisomic and euploid genes and the genetic data that support them.

With three copies of Hsa21, up to three different alleles may be present. Allelic differences on the trisomic chromosome (or more simply, a trisomic gene) may cause overexpression, underexpression, or a loss or gain of function in a particular gene product (Fig. 1A) (Ait Yahya-Graison et al., 2007). Evidence for the effects of heterotrisomy in cardiac phenotypes associated with DS has been described (Baptista et al., 2000). Many Hsa21 genes encode for transcription factors that may control the expression of other genes throughout the genome (Antonarakis, 2017). Hypothetically, different allelic combinations in a single, dosage sensitive trisomic gene may produce various expression profiles that determine whether thresholds are reached that may adversely affect particular phenotypes (Antonarakis et al., 2004) (Fig. 1A). Additionally, a particular allele of a trisomic gene may regulate another trisomic (or euploid) gene to cause a particular phenotype (Antonarakis et al., 2004, Roper and Reeves, 2006). Therefore, allelic differences in trisomic Hsa21 genes, at either a single locus or combined loci, could contribute to the presence and/or variability of specific phenotypes in DS.

Allelic differences may be important in the survival of fetuses with Ts21. The genetic burden of Ts21 leads to a fetal loss rate of up to 80% as compared to non-trisomic fetuses (Popadin et al., 2018). A “genetic handicap” model was proposed to account for fetal survival and the highly variable phenotypes in DS (Popadin et al., 2018). Slightly deleterious allelic differences throughout the genome may not play a significant role in a fetus with a normal chromosomal complement (Fig. 1B, right side). Trisomy, however, may sensitize the fetus (increase vulnerability) to these slightly deleterious variants that may occur on every chromosome and even additively combine to produce an undesirable trait (Fig. 1B, left side). Only in the presence of trisomy would these sequence variants cause an adverse developmental phenotype. By extension, fetuses with Ts21 but fewer of these variants

throughout the genome would escape embryonic lethality, whereas those with more would be miscarried (Popadin et al., 2018).

Related to the possible effects of heterotrismy and the “genetic handicap” model is the “threshold” model of sensitization and additive allelic differences (Li et al., 2012) (Fig. 1B). Trisomy may sensitize individuals to display certain phenotypes when they carry allelic variants or mutations in other genes throughout the genome; whereas individuals without trisomy would either not display the phenotype or need a higher combined number of variant alleles to display a similar phenotype. It is hypothesized that trisomy may sensitize the organism to additive deleterious allelic variations that may cause similar phenotypes in all individuals, but are more likely found in individuals with DS because of the presence of the extra Hsa21. Trisomic genes may also be affected by allelic differences in modifier genes that do not act in an additive way but may influence penetrance, expressivity, dominance or pleiotropy of a phenotype (Riordan and Nadeau, 2017, Nadeau, 2001).

Threshold model of allelic differences leading to cardiac phenotypes in DS

About 40-50% of individuals with DS have a congenital heart defect (CHD) (Freeman et al., 2008). Approximately 20% of individuals with DS have an atrioventricular septal defect (AVSD), an incidence about 2000 times the AVSD in euploid individuals (Rambo-Martin et al., 2018). Although there is an increased incidence of CHD and AVSD in individuals with DS, Ts21 by itself is not sufficient to cause cardiac deficits. For CHD, the threshold model postulates that a number of allelic variants, which alone are not sufficient to cause CHD, may additively cause AVSD associated with DS because they are sensitized by Ts21 (Li et al., 2012). Genetic variants associated with AVSD in DS have been identified on Hsa21 and throughout the rest of the genome. Yet not all individuals with sequence differences in these genes display an AVSD phenotype, either in euploid or trisomic populations. Allelic variants in *COL6A1* (*Collagen Type VI Alpha 1 Chain*) and *COL6A2* (*Collagen Type VI Alpha 2 Chain*), found on Hsa21, have been linked to AVSD in individuals with DS (Ackerman et al., 2012) (Table 1). *CRELD1* (*Cysteine Rich With EGF Like Domains 1*) mutations were identified through candidate gene screening as genetic mutations that contribute to AVSD in euploid individuals, and to a greater extent, individuals with Ts21 (Maslen et al., 2006). *FBLN2* (*Fibulin 2*), *FRZB* (*Frizzled Related Protein*), and *GATA5* (*GATA Binding Protein 5*) were ascertained as deleterious variants that may contribute to AVSD in individuals with DS (Ackerman et al., 2012). These candidate genes are related through the VEGF-A (*Vascular Endothelial Growth Factor A*) pathway, a growth factor specific for vascular endothelial cells. Additional genome wide association and copy number variant studies indicate that multiple genetic variants with a small effect size may lead to the elevated risk for AVSDs in Ts21 sensitized individuals (Ramachandran et al., 2015a, Ramachandran et al., 2015b, Rambo-Martin et al., 2018). These suggested associations encourage analyses with sufficient sample sizes of individuals with DS to identify these variants.

Allelic differences in leukemia associated with DS support the threshold model

Individuals with DS are susceptible to leukemia involving cells from the lymphoid or myeloid stem cell lineage. The presence of three copies of Hsa21 leads to a 20 fold increase in B lymphoblastic acute leukemia (DS-ALL) and a 500 fold increase in acute

megakaryoblastic leukemia (AMKL) in individuals with DS as compared to age-matched individuals with a normal chromosomal complement (Antonarakis, 2017). DS-ALL primarily occurs in Caucasians, suggesting the contribution of unique background genes in this population (Lee et al., 2016). Trisomic *HMGNI* (*High Mobility Group Nucleosome Binding Domain 1*) and *DYRK1A* (*Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A*) may cause excessive B cell proliferation (Lane et al., 2014, Thompson et al., 2015) and some “driver mutations” or allelic differences in genes such as *JAK2* (*Janus Kinase 2*), *NRAS* (*Neuroblastoma RAS Viral Oncogene Homolog*), or *KRAS* (*Kirsten Rat Sarcoma Viral Oncogene Homolog*), found in about one third of all DS-ALL, may influence susceptibility (Table 1). Somatic rearrangements, mutations in or overexpression of *CRLF2* (*Cytokine Receptor Like Factor 2*) may contribute to the occurrence of leukemia, and there is some indication that *CRLF2* and *JAK2* allelic differences may work together in DS-ALL (Lee et al., 2016). Deletions in *IKZF1* (*Ikaros Family Zinc Finger Protein 1*), *PAX5* (*Paired Box 5*), and *ETV6-IGH* (*ETS Variant 6-Immunoglobulin Heavy Locus*) rearrangements have also been found in individuals with DS-ALL and in euploid individuals (Lee et al., 2016). For DS-ALL, both heterotrissomy and trisomic sensitization to other allelic differences throughout the genome may be the genetic underpinnings for the leukemia.

Transient myeloproliferative disorder (TMD) or transient abnormal myelopoiesis (TAM), characterized by excessive clonal expansion of immature megakaryoblasts, is present in 10-15% of fetuses and infants with DS (Antonarakis, 2017, Watanabe, 2018). Truncating somatic mutations in exon 2 of the *GATA1* (*GATA Binding Protein 1*) transcription factor, found on the X chromosome, have been detected in essentially all cases of TMD and AMKL in individuals with DS (Talsma and Boyer, 2017, Taub et al., 2004). Allelic differences or mutations in *GATA1*, not always the same in individuals with DS, shorten the protein to the GATA-1s form, which, when truncated, acts as a dominant-negative and blocks normal differentiation (Yin et al., 2016) (Wechsler et al., 2002). Ts21 creates an environment, in utero, in which hematopoietic progenitor cells within the fetal liver are primed for acquisition of *GATA1* mutagenesis that reflect a “second hit,” (when combined with Ts21) thereby promoting the transient expansion of megakaryoblasts seen in TMD (Yin et al., 2016). Most cases of TMD in DS spontaneously resolve within 3 months and before one year of age, as hematopoiesis developmentally switches from fetal liver to bone marrow, and the *GATA1* megakaryoblastic clone disappears in 70% of patients with TMD. (Yin et al., 2016). Yet 20% of individuals with DS and TMD die in early infancy (Watanabe, 2018). Additionally, 20-30% of individuals with DS and TMD in infancy develop AMKL, usually before the age of 5 years (Bhatnagar et al., 2016). In contrast to individuals without Ts21, AMKL in individuals with DS is usually successfully cured with low-dose chemotherapy (Lee et al., 2016). Underscoring the requirement for trisomy in DS-AMKL, individuals without Ts21 but with germline *GATA1* mutations, analogous to those seen in TMD and DS-AMKL, have no predisposition to leukemia and *GATA1* mutations are not found in pediatric leukemia in the absence of Ts21 (Birger et al., 2013).

Individuals with DS with a silent TMD or without having TMD have developed AMKL (Watanabe, 2018). At least half of all neonates with DS and *GATA1* allelic differences have no clinical features associated with TMD, but carry the risk of subsequently developing AMKL if the mutant *GATA1* clone persists and is accompanied by other mutations. The

majority of cases of TMD resolve without sequelae as the *GATA1* mutation is lost, but ~10% of children harbor residual *GATA1*-mutant cells which then acquire transforming mutations in additional oncogenes leading to AMKL in individuals with DS (Bhatnagar et al., 2016).

Although the combination of trisomy and *GATA1* mutations are essential for TMD and DS-AMKL, allelic variations in other genes may play a necessary role in causing the leukemia. Allelic variations or mutations in genes including *FLT3* (*Fms Related Tyrosine Kinase 3*), *EZH2* (*Enhancer Of Zeste 2 Polycomb Repressive Complex 2 Subunit*), *APC* (*Adenomatous Polyposis Coli Protein*, *WNT Signaling Pathway Regulator*) *JAK* and *RAS* have been identified as causative factors in individuals with and without Ts21 that present with leukemia (Antonarakis, 2017). Additionally, trisomic genes including *RUNX1* (*Runt Related Transcription Factor 1*), *ETS* (*ETS Proto-Oncogene 1, Transcription Factor*) and *ERG* (*V-Ets Avian Erythroblastosis Virus E26 Oncogene Homolog*) may be important in TMD (Watanabe, 2018).

Taken together, the significance of allelic differences in cardiac and leukemia phenotypes and the variability of these traits in individuals with DS highlight the importance of allelic differences in trisomic genes and throughout the rest of the genome. The hypothesized genetic mechanism leading to differences in the prevalence and severity of DS traits is that the trisomic chromosome provides a sensitized state in which these allelic differences additively cause the phenotype (Li et al., 2012). This threshold model of susceptibility, illustrated in both cardiac and leukemia phenotypes, may be relevant to other phenotypes associated with DS. The threshold hypothesis also postulates that the genome can only support a limited number of genomic and/or allelic differences before adverse phenotypic changes present in the individual.

Inbred strains of mice utilized to identify allelic differences in human disorders

To understand the relationship between genes and phenotypes in health and disease, and to further investigate how allelic differences and molecular mechanisms lead to genetic disorders, inbred strains of mice are used. Inbred strains of mice are essentially clones of each other, classically defined as having undergone 20 sequential generations of brother × sister mating. Different inbred strains (e.g. C57BL/6 [B6] C3H, DBA, 129) display homozygous alleles at all genes in the genome and eliminate genetic variability in experiments using the same inbred strain (Silver, 2001), though this traditional perspective may not be universally true (Watkins-Chow and Pavan, 2008). Homozygosity for some allelic variants in inbred strains, however, may lead to detrimental phenotypes as illustrated by the blindness that develops in adult C3H mice carrying *Pde6b^{rd1/rd1}* (*Phosphodiesterase 6B*). Different inbred strains of mice may display dissimilar behavioral responses (Bouwknicht and Paylor, 2008) and even inbred substrains (e.g. B6 mice derived from a founder strain and that have been maintained separately in closed breeding colonies for many generations), may vary slightly in genetic background due to genetic drift, and may harbor allelic variations that result in diverse phenotypes (Bryant et al., 2008, Simon et al., 2013). Importantly, the magnitude of genetic contributions to behavioral phenotypes across inbred strains can be unique to the specific environment. Environmental modulation of phenotypic differences between strains was most directly shown in a study explicitly

designed to compare the degree of difference among eight inbred strains on outcomes on five behavioral tests across three laboratories while rigorously equating many environmental variables. Several behavioral traits showed similar across-laboratory outcomes with consistent strain differences, but others showed large environmental influences and less reliable strain differences across laboratories (Crabbe et al., 1999). Taken together, comparisons among inbred strains in conjunction with analyses of gene $\times$ environmental interactions (Wahlsten et al., 2003) may identify contributions of allelic differences to phenotypic variation.

Mouse models and inbred strains of mice are important contributors to understand the gene-phenotype relationship in DS. Nuances of trisomic and inbred mouse strains, if understood, may facilitate finding sequence variants that affect DS traits. Because the gene orthologues to Hsa21 are found on mouse chromosomes (Mmu)10, 16, and 17 (Reeves et al., 2001, Antonarakis et al., 2004), it is difficult, if not impossible, to construct a mouse model that replicates all the genetic and phenotypic features most often found in humans with Ts21, including an extra freely segregating chromosome. Different DS mouse models have been made and utilized throughout the years, and although none is a perfect model, the contributions of these trisomic mice help understand the gene-phenotype relationship in DS and some of the genetic differences causing the variability in occurrence and severity of DS-related phenotypes.

Breeding together mice containing duplications of the Mmu10, 16 and 17 orthologous segments generated a triple trisomic DS mouse model trisomic for all Hsa21 homologous genes, but without a freely segregating chromosome (Belichenko et al., 2015, Yu et al., 2010). Because of the difficulty in generating and maintaining triple trisomic mice, they have not been widely used in the DS community to understand the gene-phenotype relationships in DS. Different DS mouse models have been reviewed by others (Gardiner, 2015, Gupta et al., 2016, Xing et al., 2016), and only important potential allelic differences between models are outlined here (Table 2). Published data indicate that modest allelic differences in trisomic mice may significantly affect DS-related phenotypes.

Development of the Ts65Dn DS mouse model

Ts65Dn mice are the most widely used mouse model of DS, and were generated by cesium irradiation of testes of DBA/2J mice that were subsequently bred to C57BL/6J mice to recover heritable chromosomal translocations (Davisson et al., 1990, Davisson et al., 1993) (Table 2). Female mice with heterozygous reciprocal translocations were mated to (B6C3)F1 mice. Ts65Dn mice have ~93 protein coding trisomic Mmu16 genes on the small marker chromosome that includes a Mmu17 centromere with ~35 protein coding Mmu17 non-Hsa21 orthologous genes (Gupta et al., 2016, Reinholdt et al., 2011, Duchon et al., 2011). Limited efforts to breed the Ts65Dn line on to a C57BL/6J, C57BL/6JEi or C3H background were not successful. Attempts to place the T65Dn chromosome on a uniform inbred background, resulted in only 2-3 offspring, and after 2-3 backcrosses, no viable pups were born (Davisson and Costa, 1999, Paz-Miguel et al., 2001). These efforts were likely hampered by inbreeding depression and small litter sizes from Ts65Dn mice. Ts65Dn mice have been maintained as an advanced intercross line with approximately 50% B6 and 50%

C3H alleles, but also carry alleles from DBA/2J mice from which the extra chromosome was first obtained (Lorenzi et al., 2010, Reinholdt et al., 2011). This possible heterotrisomy in Ts65Dn mice may lead to allelic differences that cause phenotypic variation by a trisomic additive model to reach a particular threshold, as has been hypothesized for humans with Ts21 (Antonarakis et al., 2004). The plausible heterotrisomy, in addition to the freely segregating chromosome with an extra centromere, may account for Ts65Dn phenotypes not found in other DS mouse models with a similar trisomic genetic content (Aziz et al., 2018).

Although Ts65Dn mice are routinely maintained on a mixed background, non-Mendelian ratios between trisomic and euploid postnatal offspring demonstrate transmission distortion of the small marker chromosome (Roper et al., 2006). At embryonic days 9.5-14.5 (E9.5-14.5), no difference from Mendelian ratios of trisomic and euploid offspring was observed (Lorandean et al., 2011, Roper et al., 2006). A prenatal “genetic handicap” is not observed in Ts65Dn embryos, perhaps due to only having trisomy for about half of the Hsa21 orthologous genes. Diminished postnatal survival in Ts65Dn mice is typical, with 44% trisomic offspring observed at birth, 40% trisomic offspring at postnatal day 6, and 36% trisomic offspring at weaning (Roper et al., 2006). Failure to nurse, maternal neglect, hyperactive mothers, and cardiac deficits have been postulated to cause the reduction in trisomic mice after birth (Moore, 2006, Williams et al., 2008). Postnatal transmission distortion of the small marker chromosome may also be genetically linked to Mmu17 genes found near the centromere of the marker chromosome, or selection related to the *H2* (*Histocompatibility-2*, *MHC*) locus (Paz-Miguel et al., 2001). Ts65Dn may either carry *H2^b* (from B6) or *H2^k* (from C3H) alleles. Different studies reported a selection against progeny carrying *H2^b/H2^b* alleles (homozygous for B6 alleles at the *H2* locus) (Paz-Miguel et al., 2001) or *H2^k/H2^k* alleles (Roper et al., 2006), though the latter study was much larger than the first. Taken together, these studies suggest that allelic differences in background genes affect the survival of offspring from Ts65Dn mice.

Different laboratories have maintained Ts65Dn mice by breeding them to (B6C3)F1 or (B6EiC3Sn)F1 male mice. Transmission of the T65Dn marker chromosome at embryological, birth and weaning ages in Ts65Dn mice, however, was not significantly different in separate studies where these two different backgrounds were exclusively used (Moore, 2006, Roper et al., 2006, Williams et al., 2008). Other phenotypes have not been directly compared in Ts65Dn mice crosses with slightly different backgrounds, but it is conceivable that these small allelic differences may be a source of phenotypic variability in Ts65Dn mice. Therefore, a detailed explanation of the origin and breeding scheme of DS mouse models in written and oral presentations is necessary to begin to find influences of small allelic differences.

Comparison between Ts65Dn and B6C3.B-Ts65Dn mice

Because Ts65Dn mice are maintained as an advanced intercross through breeding to (B6EiC3Sn)F1 or (B6C3)F1 mice, they carry, on average, 50% B6 alleles and 50% C3H alleles and may be heterozygous or homozygous for B6 or C3H alleles at every locus. Unfortunately, ~25% of offspring from [Ts65Dn × (B6EiC3Sn)F1] matings are homozygous for the *Pde6b^{rd1}* allele and will show retinal degeneration and blindness in older mice. This

blindness may affect many tests of behavior, and mice homozygous for the *Pde6b^{rd1}* allele must be identified and removed from the testing cohorts. Because Ts65Dn mice are so difficult to generate (Roper et al., 2006), a new strain of Ts65Dn mice without the (*Pde6b^{rd1}*) mutation was developed (Costa et al., 2010). These mice were generated by backcrossing C3A.BLiA-*Pde6b⁺*/J mice with wild type *Pde6b⁺* to recipient C3H/HeSnJ mice for ten generations and selecting mice that carried the normal *Pde6b* allele for subsequent breeding. The resultant congenic mice with *Pde6b⁺* allele on the C3H/HeSnJ background were then intercrossed to produce C3H (C3Sn.BLiA-*Pde6b⁺*/Dn) mice homozygous for *Pde6b⁺*/*Pde6b⁺*. These mice were subsequently backcrossed to C57BL/6J mice to make an F1 hybrid B6EiC3Sn.BLi, similar to the (B6EiC3Sn)F1 hybrid routinely mated to maintain and generate Ts65Dn mice. Male B6EiC3Sn.BLi mice were mated to female Ts65Dn mice, and the trisomic female offspring were bred with B6EiC3Sn.BLi mice for an additional four generations to produce B6EiC3Sn.BLi a/A-Ts65Dn mice. All mice from B6EiC3Sn.BLi a/A-Ts65Dn × B6EiC3Sn.BLi matings will be homozygous for the *Pde6b⁺* allele and will not exhibit blindness.

When B6EiC3Sn.BLi a/A-Ts65Dn (B6C3.B-Ts65Dn) mice were compared to Ts65Dn mice, post hoc analyses found significant differences in mean body length between nose and tip of tail and between nose and base of tail in Ts65Dn and littermate control mice, but only between nose and tip of tail in B6C3.B-Ts65Dn and littermate control mice (Costa et al., 2010). Comparisons of locomotor activity, contextual and sound-cued fear conditioning, and grip forces did not significantly differ between the two strains but there were subtle differences between B6C3.B-Ts65Dn and Ts65Dn in aspects of the Morris water maze (MWM). Ts65Dn mice exhibited fewer platform crossings with a hidden probe as compared to their respective littermate controls, but the differences in the B6C3.B mice were not significant due in part to the fewer crossings by controls. Additionally there were some differences between the two trisomic strains of mice and their littermate controls in the reverse probe test of the MWM. In a single study, a few behavioral and size differences were observed between B6C3.B-Ts65Dn and Ts65Dn mice, but other phenotypes (cardiac, bone, craniofacial) have not been directly compared between these two substrains. Possible allelic and phenotypic differences between B6C3.B-Ts65Dn and Ts65Dn mice warrant precise identification of which strain of trisomic mice is used in a particular study.

Potential differences in congenic mice bred to DS mouse models

Congenic mice are produced when an allele of interest is transferred from one inbred strain to another by repeated mating and selection. (Bennett, 2000, Armstrong et al., 2006, Wakeland et al., 1997, Montagutelli, 2000). The introduction of the *Pde6b⁺* allele from donor C3A.BLiA-*Pde6b⁺*/J to recipient C3H/HeSnJ mice through ten generations of backcrossing and selection described in the paragraphs above is an example of a congenic line. With each additional backcrosses to the recipient inbred line, the donor allele number decreases, on average, by half. Ten backcross generations with selection of the allele of interest is recommended to make a congenic line and, on average, 99.9% of the resultant alleles will be from the recipient inbred line. After ten generations, the mice may be brother × sister mated to generate a mouse with homozygosity at the selected locus. Marker assisted selection of lines with fewer remaining donor alleles can be used to reduce the number of

backcross generations in “speed congenics” (Wakeland et al., 1997). With the selection of the allele of interest, there is a high probability that alleles in some regions of the genome necessary for survival or reproduction, or alleles flanking the selected gene (marker) from the donor inbred line will be carried onto the congenic strain (Wakeland et al., 1997, Montagutelli, 2000). Thus a congenic line is likely to have non-homozygous “passenger” or “hitchhiker” alleles or loci that may affect disease model genotypes (Su et al., 2009, Lusis et al., 2007, Armstrong et al., 2006). The small differences between B6C3.B-Ts65Dn and Ts65Dn mice may be due to passenger loci from one of the strains used to make the B6C3.B-Ts65Dn mice besides B6 or C3H (Montagutelli, 2000). Passenger loci may also be found when Ts65Dn mice are bred to a mouse on a similar genetic background carrying an allele for a particular gene that is knocked out to create a mouse with two copies of a particular gene on an otherwise trisomic mouse (Blazek et al., 2015a, Blazek et al., 2015b). More allelic differences will be seen throughout the genome when a congenic line that places a modified gene (gene knockout) on similar genetic background as the trisomic line is not made before mating it to a DS mouse model (Salehi et al., 2006, Garcia-Cerro et al., 2014, Garcia-Cerro et al., 2017).

The Tc1 model of DS contains an almost complete Hsa21 and cannot be inbred

The Tc1 transchromosomal DS mouse model contains a copy of almost all of Hsa21 (Table 2) and displays alterations in behavior, brain structure, heart development and craniofacial attributes (O’Doherty et al., 2005). These mice are mosaic for the Hsa21 transchromosome with a complex structure (Gribble et al., 2013). To generate Tc1 mice, MPI VI ES cells (from 129S2 mice) were injected into host blastocysts and the resultant chimeras were mated to B6 mice. The resultant offspring were mated to (C57BL/6Jx129S8)F1, BALB/c, C3H/He, and C57BL/6J mice. Only a small percentage of transchromosomal pups were transmitted from the male Tc1 parent in any of these backcrosses. When the human chromosome was transmitted through the female murine parent, 25-42% of the offspring carried Hsa21 in generations (G)2-6. When female Tc1 mice were backcrossed to BALB/c, C3H/He, and C57BL/6J mice, transmission of the extra chromosome was not found in backcross generations N3, N5 and N4, respectively. Neither Ts65Dn or Tc1 DS mice retain the extra freely segregating chromosome with limited attempts to generate an inbred line, males are relatively infertile, and the extra chromosome is passed through the female. In both of these DS models, there is a loss of trisomic offspring, and both are maintained on advanced intercross background with repeated backcrossing to B6C3F1 (Ts65Dn) or B6129S8F1 (Tc1) male mice (O’Doherty et al., 2005, Roper et al., 2006). Notably, in the aforementioned study by Crabbe et al. (1999), environmental influences on behavioral outcomes were most prominent among three genetic groups derived from the 129 strain. The allelic differences in Tc1 mice due to the transchromosome and the different genetic background (129) make it difficult to directly compare Tc1 mice to other DS mouse models.

Cardiac deficits in Ts65Dn and Tc1 mice depend on allelic differences

Cardiac deficits, especially those associated with AVSD are not seen in the numbers expected in DS mouse models to recapitulate the human phenotype (Moore, 2006). Between 8-15% of Ts65Dn mice have cardiac deficits, and types and ratios of cardiac defects seen in humans with DS are not conserved in Ts65Dn mice (Moore, 2006, Williams et al., 2008),

likely because of the different trisomic genetic content. (Moore and Roper, 2007, Williams et al., 2008). Trisomic models of DS have identified modifiers of AVSD related to DS, though only 4.7% of Ts65Dn mice have ventricular septal defects (Li et al., 2012). Ts65Dn mice on a B6N.C3H instead of a B6J.C3H background have fewer or attenuated cardiac deficits (Li et al., 2016), indicating that slight differences in background alleles in genetic substrains affect the penetrance of trisomic cardiac phenotypes. *Creld1*^{+/-} and *Hey2*^{+/-} (Hairy/enhancer-of-split related with YRPW motif) mice on a euploid background present no CHD but when bred onto Ts65Dn mice, Ts65Dn;*Creld1*^{+/-} mice had 33% and Ts65Dn;*Hey2*^{+/-} mice had 24% cardiac septal deficits. Ten percent of *Creld1*^{+/-}, *Hey2*^{+/-} compound heterozygous mice without the sensitizing trisomy had septal deficits. Septal deficits in Ts1Cje;*Creld1*^{+/-} mice were not more frequent than in Ts1Cje mice alone, even though Ts1Cje mice had significantly more septal deficits than Ts65Dn mice (Li et al., 2016). Additionally, septal defects in Ts1Rhr mice with 31 trisomic protein coding genes were not significantly different than Ts1Rhr;*Creld1*^{+/-} mice, suggesting that sensitizing trisomic genes were found on the 20% of Mmu16 found in Ts65Dn but not Ts1Cje mice. (See Table 2 for differences between these DS mouse models). Trisomic *Jam2* (*Junction adhesion molecule 2*) was identified as a potential modifier of CHD. When *Jam2* was reduced to euploid levels in Ts65Dn;*Creld1*^{+/-};*Jam2*^{+/-} mice, only 4.5% septal deficits were seen in these mice in contrast to almost 19% in Ts65Dn;*Creld1*^{+/-} littermate mice (Li et al., 2016).

When Tc1 mice were bred to B6 mice to generate embryonic progeny, 55% cardiac deficits were seen in Tc1 E14.5 embryos (as compared to 14% euploid) on a background with more B6 alleles, as compared to 38% embryonic (E14.5—as compared to 10% euploid) Tc1 cardiac deficits with a 129S8:C57BL/6J background (Dunlevy et al., 2010). Of the cardiac deficits observed in Tc1 embryos, 47% were VSD, 22% were outflow tract, and 21% were atrioventricular canal deficits. When E18.5 Tc1 embryos were examined, 21% had cardiac deficits and all embryos had a VSD. It was hypothesized that the differences between cardiac deficits in E14.5 and E18.5 Tc1 embryos with a more pronounced B6 background were due to a closing in some of the septal deficits during later development or reduced viability of embryos with more severe cardiac deficits. These cardiac deficits were likely affected by the mosaicism of Tc1 mice where 20-50% of adult hearts retain the Hsa21 transchromosome (Dunlevy et al., 2010).

The cardiac deficits in Ts65Dn and Tc1 mice demonstrate the threshold model of DS phenotypes where the trisomic chromosome acts as a sensitized background on which allelic differences additively produce CHD (Li et al., 2012). To attain the trisomic threshold, certain trisomic genes appear to be necessary because of the different CHD phenotypes seen in Ts1Rhr, Ts1Cje and Ts65Dn mice and the influence of *Jam2*. Different cardiac phenotypes may have also been influenced by ~35 trisomic non-Hsa21 Mmu17 genes in Ts65Dn mice and monosomy for ~7 Mmu12 genes in Ts1Cje mice (Duchon et al., 2011, Gupta et al., 2016, Reinholdt et al., 2011). The possible heterotrisomy in Ts65Dn and Tc1 mice may also exacerbate the cardiac (and other) phenotypes observed in these mice (Antonarakis et al., 2004).

Modeling of TMD and DS-ALL in animals and iPSCs

The effects of genes found on Hsa21 and allelic variants of other genes throughout the genome demonstrate importance in TMD and DS-ALL. An animal model of DS-ALL has been made using Ts1Rhr mice with mutation in *Jak2*, *Crlf2* overexpression, *Pax5* deficiency and a loss of *Ikzf* (Lane et al., 2014). Overexpression of trisomic *Hmgn1* in Ts1Rhr mice was suggested to suppress H3K27me3 and promote DS-ALL in vivo. *Dyrk1a*, found in three copies in both Ts1Rhr and Ts65Dn DS mice, controlled Cyclin D3 protein levels through increased phosphorylation and degradation, and impaired the cell cycle exit and proper differentiation of pre-B and pre-T cells in experiments done in euploid and an inducible *Dyrk1a* knock down mouse model (Thompson et al., 2015). Work using human induced pluripotent stem cells (iPSCs) indicates that trisomy for a 4mB region that includes the synergistic effect of *RUNX1*, *ETS2* and *ERG*, in combination with the *GATA1s* variant, is necessary to cause the hematopoietic abnormalities leading to TMD (Banno et al., 2016). The effects of other trisomic genes were also implicated in these in vitro studies. These models suggest a more defined threshold model for leukemia related to DS. The combined effect of many trisomic genes with allelic differences in euploid genes may cause DS-related leukemia. The specific genes in this enhanced threshold model are likely specific for the different types of leukemia in DS, but the combined effects of multiple trisomic genes allelic variants throughout the genome surpassing a threshold for a disease phenotype (Fig 1B) may be extended to other DS phenotypes. By using animal and cellular models of DS, such molecular mechanisms may be elucidated for other DS phenotypes.

Comparison between Ts1Rhr mice on a different genetic background

Ts1Rhr mice disproved the hypothesis that trisomy for the “Down syndrome critical region” (DSCR) comprised of ~33 genes was responsible for a subset of significant phenotypes associated with DS (Olson et al., 2004a) (Table 2). Instead of having less severe craniofacial phenotypes because of fewer genes at dosage imbalance, Ts1Rhr mice were larger than euploid littermates and had distinct craniofacial phenotypes when compared to Ts65Dn mice and their control littermates on a 50% B6 and 50% C3H genetic background. On a genetic background that was on average 50% B6, 25% C3H, and 25% 129S6, the Ts1Rhr brain size was smaller and the cerebellar size equal as compared to euploid controls, in contrast to a similar sized brain and smaller cerebellum in Ts65Dn and littermate control mice (Aldridge et al., 2007). However, on a 50% B6, 50% 129S6 background, the body and brain size of Ts1Rhr as compared to euploid controls was larger (Olson et al., 2007). Ts1Rhr as compared to euploid littermate mice also displayed a normal hippocampal size and a slightly reduced cerebellum with no significant alterations in granule or Purkinje cell densities. There were no differences in the MWM in B6.Ts1Rhr as compared to euploid littermate mice. No LTP impairment in the CA1 region of the hippocampus was detected in Ts1Rhr mice on an inbred B6 background.

Ts1Rhr mice on a similar genetic background as Ts65Dn mice (~50% B6, 50% C3H) had significantly greater body weight and brain weight and a slight but not significant increase in the size of the hippocampus (Belichenko et al., 2009). Ts1Rhr mice on this similar background also showed no deficits in spontaneous locomotion, comparable to results seen in Ts65Dn mice. Ts1Rhr, as compared to littermate controls, were more thigmotaxic in the

open field activity test, and had a lower discrimination ratio after a two-week delay in the novel object test. T maze spontaneous alteration was also significantly reduced in Ts1Rhr mice. There was also a failure to induce LTP in the fascia dentata of the hippocampus of Ts1Rhr mice on this background (Belichenko et al., 2009).

In one of the seminal investigations of background differences in DS mouse models, numerous differences in size and craniofacial precursors were seen because of allelic differences. When developmental phenotypes were examined in Ts1Rhr mice on two different genetic backgrounds, significant differences were found (Deitz and Roper, 2011) (Table 3). Trisomic and euploid E9.5 offspring from B6.Ts1Rhr (inbred) mice were significantly smaller than offspring from Ts1Rhr mice on a 50% B6, 25% C3H, and 25% 129S6 (mixed) background, though there were no significant differences in embryo volume in trisomic and euploid embryos from within a particular genetic background. In B6.Ts1Rhr embryos, first pharyngeal arch (PA1), size and number of neural crest cells, precursors to the mandible, were significantly greater in E9.5 trisomic as compared to euploid littermates, and no differences observed between trisomic and euploid PA1 on a mixed background. Additionally, RNA expression of *Ets2*, a gene in three copies in Ts1Rhr mice, was differentially expressed in the PA1 between inbred and mixed background. Although overall embryo sizes were different between the two different backcrosses of Ts1Rhr, the number of somites were not significantly different.

At P6, female trisomic B6.Ts1Rhr mice were significantly smaller than female euploid littermates, and no significant differences in the size (mass) between trisomic and euploid male inbred mice. In Ts1Rhr mice on a mixed background, there were no significant differences between female or male trisomic and euploid mice. At 28 days of age, on a B6 background, trisomic males and females were significantly smaller than euploid littermates, and Ts1Rhr males and females on a mixed background were significantly larger than euploid littermates. Taken together, these results demonstrate that different alleles may significantly affect an overall (weight or mass) or tissue-specific phenotype (size of the E9.5 PA1) and these phenotypic differences may not always be similar in size or magnitude (larger PA1 in B6.Ts1Rhr as compared to euploid embryos that show no difference in overall embryo size).

Structural and behavioral differences between Ts1Cje, Ts65Dn and Dp16 mice may be influenced by allelic differences

When compared to euploid littermates, Ts65Dn on a ~50% B6 and 50% C3H background—but not Ts1Cje or Dp16 on a B6 or ~50% B6 and 50% C3H background—displayed reduced crown-rump length, or rostrocaudal length and neocortical expansion in the brain as E15.5 embryos (Aziz et al., 2018) (See Table 2 for comparisons between these trisomic mouse strains). Deficiencies in the static and accelerating rotorod test were evident for Ts1Cje and Dp16 mice on a B6 background but not for Ts1Cje or Ts65Dn mice on an approximate 50% B6 and 50% C3H background.

In the contextual fear analysis, differences among the three trisomic models (relative to euploid littermates) were evident both during the day 1 conditioning phase and during the day 2 testing phase (Aziz et al., 2018). The B6.Ts1Cje mice had less freezing behavior than euploid controls in response to the second unsignaled shock on the training day. In the 5-

minute test of conditioned contextual fear the next day, Ts1Cje as compared to euploid mice had lower percent time freezing in the first minute but comparable freezing time for the remaining four minutes. Ts65Dn mice showed greater shock-elicited freezing than euploid littermates during the training session, but less conditioned freezing than controls in the test phase, evident particularly in the last two minutes of testing. In contrast, the Dp16 mice showed comparable amounts of freezing behavior following the unsignaled shock on the training day compared to euploid littermates, but less conditioned freezing behavior during the first two minutes on subsequent test day. These different patterns of fear-related behavior during acquisition and expression of conditioned fear across the three models suggests that the genetic differences among these three DS models are likely influencing this complex phenotype. Although not a point of focus in this report (Aziz et al., 2018), the allelic differences attributable to genetic background (B6 vs B6C3) also altered the results of Ts1Cje mice in the static and accelerating rotorod test and the contextual fear freezing test. These results could also be confounded by the extra ~35 trisomic Mmu17 genes in Ts65Dn mice and monosomy for ~7 Mmu12 genes in Ts1Cje mice (Duchon et al., 2011, Gupta et al., 2016, Reinholdt et al., 2011).

Comparisons between Ts1Cje, Ts2Cje and Ts65Dn and mice show differences between trisomic mouse models

Ts1Cje mice have a chromosomal rearrangement with the tip of Mmu16 attached to Mmu12 and therefore ~80 genes at dosage imbalance. Ts2Cje mice have similar genes at dosage imbalance as Ts65Dn mice due to a Robertsonian translocation of the T65Dn marker chromosome to the distal end of Mmu12 and are maintained on a similar background, i.e. through Ts2Cje × (C57BL/6JEi × C3H/HeSnJ)F1 matings (Table 2) (Villar et al., 2005). Male Ts2Cje mice were fertile likely due to the absence of a freely segregating marker chromosome (but only transmitted the Robertsonian chromosome 1-5% of the time, and are not routinely used to propagate Ts2Cje mice), and female Ts2Cje mice produced about twice as many trisomic offspring (43%) as Ts65Dn mice (24%) in this study. Ts2Cje mice were about 20% smaller in mass than control littermates throughout their lifetime. The weights of Ts65Dn and Ts2Cje testes were 37.9% and 65.6% smaller than control littermate testes, yet spermatogenesis was significantly altered in Ts65Dn but not Ts2Cje mice. RNA expression levels of nine trisomic genes were ~1.5 fold that of euploid levels and not significantly different between Ts65Dn and Ts2Cje mice. Similar to what had been previously observed in Ts65Dn mice (Belichenko et al., 2004), spine density but not width in the spines on dendrites in the hippocampal dentate gyrus was reduced in Ts2Cje as compared to control mice. Ts2Cje spines were also shorter and had larger heads than euploid controls, phenotypes that were comparable to those seen in Ts65Dn mice. Thus, with similar trisomic content at dosage imbalance and without an extra centromere, there are some phenotypes that are different between Ts2Cje and Ts65Dn mice.

Neurological properties were directly compared in Ts2Cje and Ts1Cje trisomic mouse models, and most of these experiments were done with both models on a C57BL/6JEi and C3H/HeSnJ hybrid background (Ishihara et al., 2010). Both Ts1Cje and Ts2Cje trisomic had smaller brains and decreased cortical neurogenesis compared to euploid littermate mice at E14.5. Three-month-old Ts1Cje and Ts2Cje mice showed ventricular enlargements and

impaired neurogenesis in the subventricular zone of the lateral ventricles and in the hippocampal dentate gyrus. However, anteroposterior brain length was significantly shorter in Ts1Cje and Ts2Cje as compared euploid littermates at 3 months age on a B6/C3H background but was not significantly shorter in Ts1Cje mice ($p=0.062$) on a B6 background at 3 months of age. Sample size may have influenced these comparisons as only three mice were sampled in each group (Ishihara et al., 2010).

Tumors, lymphoma, and amyloidosis were assessed in Ts65Dn, Ts2Cje and Ts1Cje mice (Levine et al., 2009). Differences were found in the incidence of malignant tumors and systemic amyloidosis in these strains. Yet all comparisons between Ts1Cje, Ts2Cje and Ts65Dn mice may be confounded by ~7 monosomic Mmu12 genes in Ts1Cje and ~35 Mmu17 trisomic genes in Ts2Cje and Ts65Dn mice (Duchon et al., 2011, Gupta et al., 2016, Reinholdt et al., 2011).

Conclusions

Important considerations when comparing DS mouse models

Trisomic mouse models play a key role to understand the gene-phenotype relationship in DS and to determine molecular mechanisms disrupted by trisomy. To better utilize these mouse models, and because allelic differences may determine the presence and variability of phenotypes, careful comparisons between mouse models must be made. Caveats specific to particular models should also be defined, including additional monosomic or trisomic genes, mosaicism, or missing parts of chromosomes. To limit phenotypic variability, genetic backgrounds should be matched when possible. To help understand possible contributions from allelic modifiers when direct comparisons across defined mouse models are not feasible, it is critical to include information about breeding and potential background differences. To address these issues, we suggest:

1. When phenotypes from Ts65Dn or Tc1 mice with an advanced intercross background are directly compared with other DS mouse models (often maintained on an inbred B6 background), it is desirable to have all mice on a similar genetic background to reduce effects allelic differences (Olson et al., 2004a, Starbuck et al., 2014). If comparisons must be made between mice with different genetic backgrounds, an index may be made by creating a ratio between the phenotype of the trisomic mice and euploid littermates and then comparing the ratios with each other (Olson et al., 2004b).
2. To test the influence of a trisomic gene on a DS phenotype, DS model mice are often mated to mice carrying a knockout of one copy of a particular gene, resulting in a normal copy number of the desired trisomic gene in an otherwise trisomic mouse. When these heterozygous knockout mice are mated to trisomic mice, to preserve the genetic background, it is optimal to put the mice on a similar background (e.g. B6C3F1 for Ts65Dn) before mating it to the trisomic mouse strain (Blazek et al., 2015a) or compare only mice with a similar percentage of genetic alleles from included backgrounds (Hill et al., 2009). When the genetic background is changed for a knockout, sufficient generations

of backcrossing to the new inbred strain should be done before mating to the trisomic strain to ensure that alleles from the previous background do not alter the results.

3. Acknowledge large and small genetic differences between trisomic mice in materials and methods and report differences between a specific trisomic mouse on a different background in oral and written communication. For example, differentiate the use of Ts65Dn and B6C3.B-Ts65Dn mice in the materials and methods section of the paper; there may be subtle differences in the phenotypes of these mice (Costa et al., 2010). Report results from trisomic mice on different backgrounds, whether similar or different (Aziz et al., 2018, Deitz and Roper, 2011).
4. Recognize that allelic remnants of mouse strains utilized in the generation of trisomic mouse models may still remain in the progeny of a particular model, such as the alleles from the DBA2/J mice used to generate the T65Dn marker chromosome (Lorenzi et al., 2010, Reinholdt et al., 2011) (Table 2). Possible heterotrismy in trisomic genes in DS mouse models may contribute to variation seen in a phenotype of a particular model. With the creation of a trisomic mouse model or making a congenic line, there are often passenger or hitchhiker loci that accompany the locus being selected and may alter the phenotypic variability seen in offspring from these models (Armstrong et al., 2006, Lusi et al., 2007, Montagutelli, 2000).

Acknowledging the contribution of allelic differences in trisomic and non-trisomic genes in humans with DS and DS mouse models may help better explain the phenotypic variability in DS traits. Defining these sequence differences may also be useful in understanding how DS phenotypes occur and why trisomy appears to sensitize individuals with DS to a number of phenotypic abnormalities that may not occur in individuals without Ts21. These detailed studies with trisomic individuals may also help to uncover euploid allelic differences that may be important in phenotypes of all individuals.

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Figure 1.

Allelic effects of trisomic and non-trisomic genes on DS phenotypes. The different shadings of the chromosomes represent allelic differences between genes on a chromosome. (A) A deleterious phenotype may be found when different alleles of trisomic genes only (heterotrisomy) have a combined effect that surpasses a genetic threshold. (B) A deleterious phenotype may occur when alleles of different genes on non-trisomic chromosomes combine to have a deleterious phenotype. Often the phenotype is not seen unless there is the sensitizing effect of trisomy.

Examples of trisomic and non-trisomic genes from human and mouse studies with allelic differences important in DS

Table 1:

Phenotype	Trisomic Gene	Non-trisomic Gene
AVSD	<i>COL6A1</i> (Collagen Type VI Alpha 1 Chain)	
	<i>COL6A2</i> (Collagen Type VI Alpha 2 Chain)	
	<i>Jam2</i> (Junction adhesion molecule 2)	
		<i>CRELD1</i> (Cysteine Rich With EGF Like Domains 1)
		<i>FBLN2</i> (Fibulin 2)
		<i>FRZB</i> (Frizzled Related Protein)
		<i>GATA5</i> (GATA Binding Protein 5)
		<i>Hey2</i> ^{+/−} (Hairy/enhancer-of-split related with YRPW motif)
	<i>HMGNI</i> (High Mobility Group Nucleosome Binding Domain 1)	
DS-ALL	<i>DYRK1A</i> (Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A)	
		<i>JAK2</i> (Janus Kinase 2)
		<i>NRAS</i> (Neuroblastoma RAS Viral Oncogene Homolog)
		<i>KRAS</i> (Kirsten Rat Sarcoma Viral Oncogene Homolog)
		<i>CRLF2</i> (Cytokine Receptor Like Factor 2)
		<i>IKZF1</i> (Ikaros Family Zinc Finger Protein 1)
		<i>PAX5</i> (Paired Box 5)
		<i>ETV6-IGH</i> (ETS Variant 6-Immunoglobulin Heavy Locus)
TMD/AMKL	<i>RUNX1</i> (Runt Related Transcription Factor 1)	
	<i>ETS</i> (ETS Proto-Oncogene 1, Transcription Factor)	
	<i>ERG</i> (V-Ets Avian Erythroblastosis Virus E26 Oncogene Homolog)	
		<i>GATA1</i> (GATA Binding Protein 1)
		<i>FLT3</i> (Fms Related Tyrosine Kinase 3)
		<i>EZH2</i> (Enhancer Of Zeste 2 Polycomb Repressive Complex 2 Subunit)
		<i>APC</i> (Adenomatous Polyposis Coli Protein, WNT Signaling Pathway Regulator)
		<i>JAK</i> (Janus Kinase)
		<i>RAS</i> (Rat Sarcoma Viral Oncogene Homolog)

Table 2: Selected Down syndrome mouse models and how they were made, including relevant inbred strains

Common name	Gene/allelic symbol	Derivation	Mb at dosage imbalance	Trisomic genes	Protein coding genes at dosage imbalance	Mouse strains of origin	Reference
Ts65Dn	Ts(17 ¹⁶)65Dn	Radiation induced, Chr 16 translocation to Chr 17	16.4	104	93	DBA/2J × (B6 × C3H)F1 Freely segregating marker chromosome	(Davisson et al., 1990, Davisson et al., 1993)
Tc1	Tc(HSA21)1TybEmcf	Irradiated microcell-mediated chromosome transfer	42	200	129	129S2/SvPas × (C57BL/6J × 129S8)F1 Transchromosomal for ~90% of Hsa21	(O'Doherty et al., 2005)
Ts1Cje	Ts(16C-tel)1Cje	Spontaneous, reciprocal translocation, Mmu12 and 16	10.3	94	74	C57BL/6J × (Rb(11.16)2H × Rb(16.17)32Lub)F1 Two normal Chr 16s and the reciprocal translocation product 12 ¹⁶	(Sago et al., 1998)
Ts2Cje	Ts[Rb(12.1716)]2Cje	Spontaneous Robertsonian fusion between T(17 ⁽¹⁶⁾)65Dn and Mmu12	16.4	104	93	Rb(12.17 ¹⁶)2Cje × (C57BL/6J × C3H/HeSn)F1	(Villar et al., 2005)
Ts1Rhr	Dp(16Chr1-Fam3b)1Rhr	Duplication using Cre/loxP-mediated recombination	5.3	33	31	129S6/SvEvTac × (B6 × C3H)F1	(Olson et al., 2004a)
Dp(16)1Yey	Dp(16Lipi-Zbtb21)1Yey	Duplication using Cre/loxP-mediated recombination	27.9	113	105	129S7/SvEvBrd-Hprt ^{b-m2} × C57BL/6J	(Li et al., 2007)
B6C3.B-Ts65Dn	B6EiC3Sn.BLiA-Ts(17 ¹⁶)65Dn/Dnl	Ts65Dn mice bred to congenic mice to eliminate the <i>Pde6rd</i> allele	16.4	104	93		(Costa et al., 2010)
Dp(17)3Yey	Dp(17Abcg1-Rtp1b)3Yey	Duplication using Cre/loxP-mediated recombination	1.7	19	21	129S7/SvEvBrd-Hprt ^{b-m2} × C57BL/6J	(Yu et al., 2010)
Dp(10)2Yey	Dp(10Pmt2-Pdxk)2Yey	Duplication using Cre/loxP-mediated recombination	2.9	41	37	129S7/SvEvBrd-Hprt ^{b-m2} × C57BL/6J	(Yu et al., 2010)

Data from (Gupta et al., 2016), The Jackson Laboratory <https://www.jax.org>, and references shown.

Table 3:
Phenotypic differences in development of Ts1Rhr mice on different genetic backgrounds

	B6 (inbred)	~50% B6, 25% C3H, 25% 129 (mixed)	Comparison between all offspring from inbred and mixed backgrounds
E9.5 PA1 volume	Trisomic > euploid	No difference	Inbred < mixed
E9.5 PA1 neural crest number	Trisomic > euploid	Not done	
Somite stage	No difference	No difference	
E9.5 embryo body size	No difference	No difference	Inbred < mixed
E9.5 litter size			No difference
E9.5 % trisomic offspring			Inbred < mixed
P6 female weight	Trisomic < euploid	No difference	
P6 male weight	No difference	No difference	
P6 litter size			No difference
P6 % trisomic offspring			No difference
P28 female weight	Trisomic < euploid	Trisomic > euploid	
P28 male weight	Trisomic < euploid	Trisomic > euploid	
<i>Dyrk1a</i> RNA expression	No difference	No difference	
<i>Rcan1</i> RNA expression	No difference	No difference	
<i>Eis2</i> RNA expression	Trisomic < euploid	No difference	