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Mouse models of Down syndrome: gene content and consequences

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Abstract

Down syndrome (DS), trisomy of human chromosome 21 (Hsa21), is challenging to model in mice. Not only is it a contiguous gene syndrome spanning 35 Mb of the long arm of Hsa21, but orthologs of Hsa21 genes map to segments of three mouse chromosomes, Mmu16, Mmu17 and Mmu10. The Ts65Dn was the first viable segmental trisomy mouse model for DS; it is a partial trisomy currently popular in preclinical evaluations of drugs for cognition in DS. Limitations of the Ts65Dn are: (i) it is trisomic for 125 human protein coding orthologs, but only 90 of these are Hsa21 orthologs; and (ii) it lacks trisomy for ~75 Hsa21 orthologs. In recent years, several additional mouse models of DS have been generated, each trisomic for a different subset of Hsa21 genes or their orthologs. To best exploit these models and interpret results obtained with them, prior to proposing clinical trials, an understanding of their trisomic gene content, relative to full trisomy 21, is necessary. Here we first review functional information on Hsa21 protein coding genes and the more recent annotation of a large number of functional RNA genes. We then discuss the conservation and genomic distribution of Hsa21 orthologs in the mouse genome and the distribution of mouse-specific genes. Lastly, we consider the strengths and weaknesses of mouse models of DS based on the number and nature of the Hsa21 orthologs that are, and are not, trisomic in each, and discuss their validity for use in preclinical evaluations of drug responses.

Introduction

Down syndrome (DS) is caused by trisomy of human chromosome 21 (Hsa21) and the increased expression, due to dosage, of some subset of the encoded genes. The phenotype of DS includes variable severity of developmental perturbations that affect most organs and organ systems (Capone 2001; Antonarakis et al 2004). DS is the most common genetic cause of intellectual disability (ID), and although ID can be mild, the average IQ is in the range of 40–50 (Canfield et al 2006; Irving et al 2008; Chapman and Hesketh 2000). DS is also the most common genetic cause of Alzheimer's Disease (AD): all individuals with DS

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develop the neuropathology of AD by the age of 30–40, and half will progress to an AD-like dementia by the age of 60 (Head et al 2016). Because of its frequency, at one in approximately 1000 live births worldwide (Canfield et al 2006; Irving et al 2008), DS is a focus of much research.

As with the study of most human diseases, mouse has played a prominent role among model systems. DS, however, is more complicated to model adequately in mouse than many human conditions. DS is a contiguous gene syndrome that spans the entire ~35 Mb of the long arm of Hsa21. The concept of a “Down Syndrome Critical Region” (DSCR), defined as the minimal region of overlap among a small number of people with DS due to partial trisomy Hsa21 (Rahmani et al. 1990), was introduced as a region of Hsa21 containing genes essential for the DS phenotype. It was controversial when proposed (Korenberg et al. 1994) and has been discredited: trisomy of other segments of Hsa21 not overlapping with the DSCR are associated with a diagnosis of DS, including ID (Korenberg et al. 1994; Nelson and Gibbs 2004; Korbelt et al. 2009). Thus, genes distributed throughout Hsa21q remain of potential relevance to DS features: it is the function of a Hsa21 gene and its elevated expression in DS that dictates its potential relevance, not its location within Hsa21. For a small number of Hsa21 genes, detailed functional analyses strongly suggest their contributions to the DS phenotype (reviewed in Sturgeon et al 2012). However, for the majority, functional information is limited or non-existent and, as a result, no gene and no segment of the long arm of Hsa21 (Hsa21q) can be excluded from possible contributions.

Regions of Hsa21q synteny in mouse involve segments of three chromosomes, the ~28 Mb of the telomeric region of mouse chromosome (Mmu) 16, an internal ~1.5 Mb segment of Mmu17 and an internal ~3 Mb segment of Mmu10 (Davisson et al 2001). From a genetic perspective, a complete mouse model of DS would be trisomic for all three segments. However, while many mouse models have been constructed, none is trisomic for all Hsa21 orthologs, and breeding to obtain a complete trisomy is complicated. The oldest and as a result, the most popular mouse model of DS is the Ts65Dn (Davisson et al. 1990, 1993; Reeves et al 1995). It is trisomic for ~55% of Hsa21 orthologous protein coding genes and is also trisomic for a large number of genes that are not orthologs of Hsa21 proteins (Duchon et al 2011); they map instead to Hsa6 and thus are not relevant to DS and may confound phenotypic consequences. The Ts65Dn does, however, indeed display many features relevant to DS, including abnormalities in neuronal number and morphology in some brain regions and deficits in learning and memory (reviewed in Rueda et al. 2012). Over the last few years, numerous drugs/small molecules have been shown to rescue one or more abnormalities in the Ts65Dn (reviewed in Gardiner 2014) and these successes have led to considerable enthusiasm for clinical trials. The Ts65Dn is, however, the only DS model so far used in preclinical assessments of drugs for cognition in DS. This leaves untested the effect on phenotype, and drug responses, of trisomy of the remaining Hsa21 genes.

There has been much discussion in the scientific literature recently regarding the failure rate (>80%) of clinical trials that are based on preclinical evaluations in mouse models (Perrin 2014; McGonigle and Ruggeri 2014). This is true in a broad range of diseases, e.g. cancer, disorders of the central nervous system, inflammation, Fragile X, etc. (Begley and Ellis 2012; McGonigle and Ruggeri 2014, McGonigle 2014). Clinical trials are expensive and not

without a toll on participants. There should, therefore, be reasoned concern regarding clinical trials for cognition in DS and this concern should include critical consideration of the genetic validity of the mouse models used in preclinical studies.

The goal of this work is to review the gene content of Hsa21 and clarify the number, nature and distribution of Hsa21 orthologs in the mouse genome. To aid in the design, and the critical interpretation of results, of preclinical experiments in DS mouse models, we first discuss current information on Hsa21 classical protein coding genes and new facts regarding long non-coding RNA genes. We then discuss the conservation of these genes in mouse, species-specific genes and the trisomic gene content of several mouse models. The goal is to provide the interested researcher with sufficient information to judge the appropriate use, and the abuse, of data obtained with each model. We start, however, with a review of the basic elements of mammalian genomic sequence annotation for gene content, how these data are curated and where they are available, to make clear how genes are identified and the confidence of these identifications.

Overview of genomic sequence annotation for gene content

Gene identification is still strongly based on experimental observation, i.e. detection of transcripts, by sequencing of cDNA libraries or RNAseq, and/or by targeted approaches of RT-PCR and RACE. Extensive contributions to mammalian transcript catalogs came first from the database of expressed sequence tags (dbEST), and then from the Functional Annotation of the Mammalian Genome (FANTOM) and the Encyclopedia of DNA Elements (ENCODE) projects (reviewed in de Hoon et al. 2015; and Harrow et al. 2012, Derrien et al. 2012). Both FANTOM and ENCODE involve, not only large scale experimental efforts, but also extensive manual curation, and together have produced annotations of gene structures, including experimentally verified 5' and 3' ends, alternative splice variants and predicted coding capacities, for both human and mouse. Additional information includes identification of patterns in transcription factor binding and DNase hypersensitivity sites, histone modifications and CpG islands.

Determination of the gene content of any human genomic segment is now based on integration and graphical representation of comprehensive experimental datasets that include dbEST, FANTOM, and GENCODE, among other datasets, and application of well-established bioinformatics tools. Two popular public annotation databases are the Genome Browser at the University of California Santa Cruz (UCSC; <http://genome.ucsc.edu/>; Rosenbloom et al. 2015) and the Ensembl Genome Browser (<http://www.ensembl.org/index.html> Flicek et al 2014) supported by the European Bioinformatics Institute (EBI) at the Sanger Institute. Both annotate the same genomic sequences and the automated annotation tools provide similar functions. Although the formats differ, both browsers provide graphical displays of largely the same comprehensive information regarding gene structures and organization and both provide gene information for download.

The Entrez Gene database at the National Center for Biotechnology Information (NCBI) in the US provides, in part, Reference Sequences (RefSeq) for mRNA, protein and functional RNAs (O'Leary et al 2016). These consist of gene products for which supporting evidence

has been manually reviewed, and are designed, as the name implies, to serve as reference sequences for each annotated gene and its splice variants. The Vertebrate Genome Annotation (VEGA) database (<http://vega.sanger.ac.uk>) provides manually reviewed and curated gene structures for whole chromosomes/chromosomal regions, calling protein coding, non-coding RNA transcripts, and pseudogenes; it is uniquely updated through the Human and Vertebrate Analysis and Annotation (HAVANA) (Harrow et al. 2014). The HUGO Gene Nomenclature Committee (HGNC) at EBI assigns standardized names and descriptions to human genes, both protein coding and functional RNA, that have passed specific validation tests (Gray et al 2015). UCSC, RefSeq, Ensembl, VEGA and HGNC gene annotations all can be found in both the UCSC and the Ensembl browsers.

As the GENCODE and FANTOM projects matured, an important new genomic feature became evident: the presence of large numbers of transcripts lacking obvious protein coding characteristics (Mattick 2003; Mattick and Makunin 2006). These transcripts were found to encode no orfs or orfs less than 100 amino acids in length, frequently lacking evolutionary conservation, and typically showing low levels of expression often restricted in time and place. There was considerable debate about the functional significance of these transcripts and suggestions that they were transcriptional noise (Louro et al 2009; Huttenhofer et al 2005). However, intense study over the last several years has revealed a remarkable repertoire of biological functions among individual long non-coding RNAs (lncRNAs). The versatility in function arises in part because, as RNA molecules, the primary nucleotide sequences and/or associated secondary or tertiary structures can allow lncRNAs to recognize and bind to specific DNA or RNA sequences and/or to individual proteins or protein complexes (Bergmann and Spector 2014). Some lncRNAs have been shown to regulate transcription of genes either neighboring their site of synthesis or at distal locations through interactions with chromatin (Vance and Ponting 2014). In stem cell biology, lncRNAs have been shown to regulate genes involved in neurogenesis, epidermal differentiation, and myoblast progenitors (Ng et al 2013; Ramos et al 2015; Lopez-Pajares et al 2015; Ballarino et al 2015), and to participate directly in regulation of the pluripotent state in embryonal stem cells (Flynn and Chang 2014). Some lncRNAs function as tumor suppressors, others as oncogenes, and some alter the stability of mRNAs or the efficiency of translation (Zhou et al 2012; Gupta et al 2010; Mercer et al. 2009). On the whole organism scale, critical functions of several lncRNAs were demonstrated in individual knockout mice, where phenotypes included embryonic lethality (Sauvageau et al. 2013; Li and Chang 2014; Goff et al. 2015).

The proportion of lncRNA genes with assigned function remains small because critical sequence features are unknown and analysis is not so far amenable to high throughput techniques. In addition, even predicting a gene as a functional non-coding RNA versus protein coding remains challenging and lacks absolute criteria. Short orf proteins exist; several hundred human proteins less than 100 amino acids in length are annotated in SwissProt, among them the Hsa21-encoded proteins, SMIM11 and PCP4 (58 and 62 amino acids, respectively) and S100B, CSTB and SUMO3 (each 92–98 amino acids). Larger scale identification of functional peptides translated from short orfs has been reported (reviewed in Andrews and Rothnagel 2014; Saghatelian and Couso 2015), although criteria for mass spectrometric validation of short orfs are still evolving (Bruford et al 2015). In spite of the challenges in identification and functional determination, it is now generally accepted that

the human genome contains large numbers of lncRNAs. Indeed, Derrien et al. (2012) reported >9000 lncRNA genes in their review of the GENCODE project within ENCODE.

With these advances in transcript identification and characterization, the number of Hsa21 genes has grown accordingly. When the genomic sequence of Hsa21 was first reported in 2000 (Hattori et al. 2000), 225 genes/gene models were annotated on 21q. Over the next ten years, the number of gene structures grew to >500 (Sturgeon and Gardiner 2011) and now stands at >550 (12–2015) in VEGA. The increases are in genes lacking sequence similarities to genes of known functions, encoding ambiguous and short orfs, and classed as lncRNA genes. Given these increases, it is not surprising that, in spite of genomic resources and numerous databases, it has become time consuming for a DS researcher to understand the criteria for gene annotation and to evaluate accurately the resulting gene content of Hsa21 and of mouse models of DS. In the following sections, we discuss protein coding gene content and then lncRNA genes within Hsa21.

Protein coding gene content of Hsa21

VEGA currently annotates 218 protein coding genes on the long arm of Hsa21 (21q) and 15 on the short arm (21p) (Table 1). For considering possible contributions to phenotypic features in DS and in partially trisomic mouse models, we confine the discussion to genes on 21q. It is helpful to classify these proteins based on functional properties and by distribution within Hsa21. Forty-nine genes within 21q encode members of the large family of keratin-associated proteins, KRTAPs. These genes are present in two clusters, one of 33 genes within 21q22.11 and the other of 16 within 21q22.3. The majority of these genes are intronless and a number of KRTAP pseudogenes are present within both clusters. Other KRTAP gene clusters are present on human chromosomes 11 and 17. KRTAPs are restricted in expression and in function, with their role to provide structure and stability to hair fibers. It seems unlikely that these genes are driving important phenotypic features in DS (Rogers et al. 2002, 2006). Therefore, if the goal is to understand how trisomy of Hsa21 genes contributes to features of the DS phenotype, then including KRTAPs without qualification in a gene count artificially inflates the number of genes requiring thoughtful analysis. We, therefore, confine further discussion of protein coding genes to non-KRTAP genes.

Of the remaining 169 protein coding genes within 21q, five are classed as novel proteins; the amino acid sequences of these proteins show no similarities to any non-primate proteins and no domains with functional annotation or recognizable amino acid patterns. This leaves 164 protein coding genes with some level of functional annotation. It is noteworthy that the large majority of these genes were included in the original annotation of Hsa21 (Hattori et al. 2000; Sturgeon and Gardiner 2011).

As one would expect, these 164 genes are diverse in their functional properties (Supplemental Table S1). Among them are 17 transcription factors/modulators, eight proteins functioning in the ubiquitin/proteasome pathway, seven involved in RNA processing, and eight relevant to Alzheimer's Disease, including the amyloid precursor protein, APP, and seven proteins that modulate APP processing or trafficking (Figure 1). Thirty-four 21q proteins are annotated in OMIM for mutations causing Mendelian disorders

in the human population, including five that cause intellectual disability (Supplemental Table S2).

Figure 1 shows the distribution of protein coding genes within Hsa21q, and the distribution of some specific functional categories, with respect to the regions of synteny within Mmu16, 17 and 10. Two features of the distribution of Hsa21 protein coding genes are important to note: (i) in spite of the relatively large size of the Mmu16 syntenic region, spanning ~30 of the 35 Mb of Hsa21q, it contains only 65% of these protein coding genes, with the other 35% contained within the ~4 Mb of the Mmu17 and Mmu10 syntenic regions, and (ii) proteins from each of the illustrative functional categories are found within each of the three mouse syntenic regions.

Of the 15 protein coding genes on 21p, 11 are identical in nucleotide sequence and structure to genes established to map to 21q22. These include contigs of 4 genes (CBS through CRYAA) and 5 genes (TRAPPC10 through DNMT3L) that map within 21q22.3 and adjacent genes SMIM11 and KCNE1 within 21q22.1. Further investigation is required to determine if the copies of these genes mapped to 21p are bona fide duplications or (more likely) arise from clone or mapping artifacts.

Non-coding gene content of Hsa21q

Among the >9000 human lncRNA genes reported by Derrien et al. (2012), 225 mapped to Hsa21. VEGA currently annotates 280 non-coding genes within 21q. These include 47 that are antisense to protein coding genes, 5 microRNAs and 228 other lncRNAs. The RFAM database annotates a further 24 putative microRNA genes. (VEGA annotates 45 lncRNA genes within 21p; several map with ~100% identity to the same regions of 21q as the 11 protein coding genes duplicated on 21p. We do not discuss 21p lncRNA genes further.)

Experimental demonstration of functions of Hsa21 non-coding genes is decidedly limited but nevertheless serves to indicate the potential importance of such genes to the DS phenotype. The MIR155 (originally, BIC) gene has been studied for roles in lymphoma (reviewed in Lawrie 2013; Faraoni et al. 2009), in mitochondrial function, in AD and inflammation and in regulation of a number of neurologically relevant target genes (Quiñones-Lombraña and Blanco 2015; Song and Lee 2015; Bofill-De Ros et al. 2015). The MIR99AHG, or MicroRNA 99A host gene, contains three microRNA genes within its introns: mir99a, let7c and 125b2. Each of these microRNA genes has been observed to be upregulated in a diverse set of malignancies. MIR99A has been shown to repress metastasis in several cancer types and to inhibit MTOR pathway signaling (Wu et al 2015; Yu et al 2015; Yang et al 2014; Warth et al 2015). MIRLET7c has been reported to suppress cell growth, migration and invasion in nasopharyngeal and non-small cell lung cancer (Liu et al 2014; Zhao et al 2014). Data for MIR125b2 are less clear because reports often do not discriminate between 125b1 and 125b2, however, MIR125b has been reported to promote glioblastoma cell growth but to suppress breast cancer growth (Wu et al 2013; Feliciano et al 2013). Although additional functions likely will be discovered for each of these microRNAs, it is already clear that they contribute to normal processes; contributions to aspects of the DS phenotype, when they are over expressed, cannot be discounted.

The mature spliced transcript of the host MIR99AHG gene also requires consideration. Originally named C21orf34/35, it was shown to be composed of >11 exons, spanning >500 kb, and subject to considerable alternative splicing, although no obvious orf was found except in the 5'-most exons (Gardiner et al. 2002). Recently, a role for this transcript, alias MONC, in mediating acute megakaryoblastic leukemia (AMKL) was demonstrated (Emmrich et al. 2014). This is of interest because of the increased incidence of AMKL in DS, where MONC is elevated in expression. The organization of MONC/Mir99AHG as a functional lncRNA and not just a host gene for intronic small RNA genes is not unique in the human genome. The GAS5 (Growth Arrest-specific 5) gene, that maps to Hsa1, is also a spliced transcript that contains several small RNA genes (small nucleolar RNA, snoRNAs) within its introns (Smith and Steitz 1998). Within the mature, spliced host GAS5 transcript, a region of secondary structure binds to and represses the activity of the glucocorticoid receptor, thus regulating metabolic levels and cell survival. More recently, the GAS5 spliced transcript has been shown to function as a tumor suppressor, and to have diagnostic potential in multiple malignancy types (Yu and Li 2015). Given such examples, it is conceivable that some of the many other Hsa21 lncRNA genes also have complex functional properties.

One last example of an RNA gene from Hsa21 involves an enhancer RNA (eRNA) associated with the nuclear hormone repressor interacting protein, NRIP1, that maps in proximal 21q21. Treatment of a breast cancer cell line with estradiol (E2) was shown to induce expression of a number of protein coding genes and their associated upstream enhancer eRNAs. Among those chosen for detailed analysis was the NRIP1 eRNA that was shown to promote an intra-Hsa21 chromatin interaction between the NRIP1 locus in proximal 21q21 and that of the trefoil factor gene, TFF1, located within 21q22.3. Inhibition of eNRIP1 perturbed expression responses of both NRIP1 and TFF1 to estrogen (Li et al., 2013).

Expression patterns of lncRNAs are robustly reproducible and the current Hsa21 gene annotation is likely to be substantially correct. Hsa21 therefore encodes more RNA genes than protein coding and it remains quite incorrect to state, without qualification, the gene content of Hsa21 by referring only to 222 protein coding genes. Although data are limited, examples of functions of non-coding genes (on Hsa21 or elsewhere) indicate that much of importance to understanding the development of the DS phenotype may lie within overexpression of some of the hundreds of lncRNA genes encoded by Hsa21.

Number of genes in Hsa21 mouse syntenic regions

Conservation in mouse is not a prerequisite in the VEGA annotation system for calling a human gene structure. VEGA annotation for the mouse genome is not complete and does not include the entireties of the Hsa21 syntenic regions. Accordingly, for this discussion, gene content of mouse Hsa21 regions of synteny has been determined from data present in the UCSC Genome Browser within RefSeq and Encode/Gencode tracks, plus manual assessments.

Among protein coding genes, the two clusters of KRTAP genes seen on Hsa21 are present in mouse, one cluster on Mmu16 and the other on Mmu10. Further experiments and curation

are needed for an accurate count of members in each cluster, but at 37 and 13, respectively, the numbers are similar to those on Hsa21q. Excluding KRTAPs, an additional 166 diverse protein coding genes are annotated within the mouse regions and, of these, 158 have orthologs on Hsa21, with the distribution shown in Figure 1: 102 on Mmu16, and 19 and 37 on Mmu17 and Mmu10, respectively. Mouse-specific protein coding genes are the integrin beta 2-like gene, ITGB2L, and three transcripts encoding uncharacterized proteins, all located within the Mmu16 region. Conversely, Hsa21 protein coding genes lacking orthologous mouse genes expected within the Mmu16 syntenic region include POTED (prostate, ovary, testis, and placenta expressed ankyrin domain family member D; Bera et al. 2002; located centromere proximal to the Mmu16 syntenic region), the T-complex-like-10 (TCP10L), the DS Critical Region 4 (DSCR4; Nakamura et al. 1997) that is expressed in placenta, testis and connective tissue, and PLAC4 that has highest but not exclusive expression in placenta (Kido et al. 1993), plus five novel genes.

Among non-coding genes, the five Hsa21 microRNAs, including the Mir155/Bic gene, are conserved in mouse at the orthologous genomic positions relative to protein coding genes. The mouse Mir99ahg/C21orf34 gene hosts the orthologous microRNA genes and resembles the human gene in complexity, spanning >500 kb and encompassing 10–12 exons that show considerable alternative splicing (Gardiner et al 2002). Nucleotide sequences of some exons are conserved, although at lower levels than orthologous protein coding genes.

lncRNA genes are equally prevalent in mouse, but few are recognizably conserved at the nucleotide level (Table 2, Supplementary Table S3; Sturgeon and Gardiner 2011). This does not mean that functions of these genes are not conserved. Functions could be dictated by conserved nucleotide segments that are much shorter and more subtle than those seen in classical protein coding genes (Mercer et al. 2009), or by genomic positions conserved relative to coding or regulatory sequences of target genes. As illustration, many gene structures on Hsa21 were identified as antisense to RefSeq protein coding genes (Sturgeon and Gardiner 2011); mouse orthologs of many of these RefSeq genes are also associated with antisense transcripts. Alternatively of course, lncRNA functions may be governed by conserved secondary or tertiary structures. With the expanding information regarding lncRNAs, it is unreasonable to exclude genes from consideration simply based on lack of obvious nucleotide conservation between human and mouse.

To reiterate an important message from these annotations: the number of genes annotated within any segment of Hsa21 is not necessarily the number of genes annotated in the syntenic mouse region, and it is certainly not the number of genes recognizably conserved in the orthologous mouse region. While it is easier for the DS researcher to focus studies on the 158 diverse protein coding genes on Hsa21 that are conserved in syntenic mouse genomic regions, to do so may not lead to the necessary understanding of the molecular basis of DS phenotypic features, nor to a reliable determination of the validity of mouse models of DS.

Mouse models of DS and trisomic gene content

Figure 2 shows the regions of Hsa21 synteny that are trisomic in several DS models. The majority of these models are trisomic for sub regions of the Mmu16 syntenic segment. This

is not due to any knowledge that genes on Mmu16 individually or collectively are of any greater biological import for the DS phenotype than those on Mmu17 or Mmu10, but rather it is due to the chromosomal location and relative size of syntenic regions. On Mmu16, the syntenic segment is the telomere proximal region, while those on Mmu17 and Mmu10 are internal (Davisson et al 2001). The Mmu16 telomeric location facilitated the creation of the Ts65Dn: testis of male mice were irradiated to fragment chromosomes; offspring of breeding these mice to unirradiated females were screened cytogenetically to identify those carrying chromosomal rearrangements that produced trisomy of the telomere of Mmu16 (Davisson et al. 1990). The resulting Ts65Dn contains a marker chromosome composed of the majority of the Mmu16 syntenic segment translocated to the pericentromeric region of Mmu17 (Davisson et al. 1990, 1993; Reeves et al. 1995). Application of the same technique would not have been practical for precision generation and identification of trisomies of the small internal Mmu17 and Mmu10 syntenic segments. Also, because variations in gene density were not well documented at that time (which was prior to availability of whole genome sequence), the Mmu16 region, at ~25 Mb (vs. ~1–3.0 Mb for the Mmu17 and Mmu10 segments), was presumed to encompass the large majority of Hsa21 orthologs. In fact, the Ts65Dn is trisomic for only 90 of the 158 non-KRTAP Hsa21 protein coding orthologs. In addition, the assumption that the Mmu17 pericentric region, syntenic to Hsa6 and trisomic in the Ts65Dn, could be ignored because of its small size was also incorrect. In fact, this region was recently shown to encode 35 protein coding and 15 lncRNA genes (prior reports of 60 genes included ten pseudogenes; Duchon et al 2011), i.e. approximately one quarter of the protein coding genes trisomic in the Ts65Dn are not Hsa21 orthologs. These genes are discussed further below.

The publication of the Ts65Dn provided the first viable segmental trisomy mouse model of DS and served to focus research on the Mmu16 region. Additional mouse models, the Ts1Cje and the Ts1Rhr, are trisomic for subsets of genes trisomic in the Ts65Dn (Sago et al 1998; Olsen et al 2004). The Ts1Rhr was the first DS model created using the Cre/lox technique of chromosomal engineering and was designed to replicate trisomy of the DSCR (Olson et al. 2004). It became of particular interest because it demonstrated that trisomy of this region was not sufficient to replicate all structural and functional abnormalities seen in the Ts65Dn, further discrediting the concept of the DSCR (Olson et al. 2004, 2007). Chromosomal engineering was next successfully used to create the Dp(16)1Yey (abbreviated Dp16) carrying a duplication of the entire Mmu16 syntenic segment (Li et al. 2007). Subsequently, models were generated with trisomy of partial and complete syntenic segments of Mmu17, respectively the Ts1Yah (Pereira et al 2009) and the Dp(17)1Yey (abbreviated Dp17; Yu et al 2010b), the complete Mmu10 segment, the Dp(10)1Yey (abbreviated Dp10), and an additional sub region of Mmu16, the Ts3Yah (Brault et al 2015). Unique among mouse models of DS is the Tc1, not trisomic for orthologous mouse regions, but instead carrying a human chromosome 21. While first reported to be an almost complete Hsa21q (O'Doherty et al. 2005), subsequent DNA sequencing revealed numerous deletions and rearrangements within the chromosome, caused by methods used in construction and leaving intact and functional only 125 of the 158 orthologs (plus KRTAP genes) (Gribble et al. 2013). In addition, being a chromosome with a human centromere in a mouse

background, the Tc1 mice are mosaics so that a variable number of cells in any tissue carry the Hsa21q.

Table 2 summarizes the conserved non-KRTAP protein coding gene and RNA gene content of the major models shown in Figure 2. Details on phenotypic features are available in recent reviews (Rueda et al. 2012; Choong et al. 2015).

Gene-phenotype correlations

Identifying specific genes that, when trisomic, cause individual DS phenotypic features has long been a goal of many researchers in the field. However, the rarity of individuals with DS due to partial trisomy Hsa21, and the lack of uniform comprehensive phenotypic determinations, has hampered progress. The availability of mouse models, segmental trisomies as shown in Figure 2 and single gene transgenics and knockouts, offers the potential to pursue this effort in mice. While the concept is simple, in practice, both the experiments and the interpretation of results are complex. To be relevant to DS, gene-phenotype correlations established in mouse models must be evaluated in the context of functional information for all genes that are trisomic in the model and all Hsa21 genes/orthologs that are not trisomic, plus data available from other DS models. It is important to recognize that gene-phenotype correlations can depend on genomic context: there are functional interactions among Hsa21 orthologs, some that are not in close physical proximity and/or not on the same mouse chromosome or trisomic in the same DS mouse model. Examples of context-specific trisomic gene consequences are listed in Table 3 and discussed here.

(i) Effects of trisomy of *Dyrk1a* are context specific

The protein kinase, *Dyrk1a*, is the most popular Hsa21 gene in the study of DS (Becker et al 2015). Among its known substrates are transcription factors, splicing factors, endocytic scaffolding proteins, and Hsa21-encoded proteins. Transgenic mice overexpressing *Dyrk1a* from human or mouse genomic constructs display, among other features, deficits in the Morris water maze (MWM) (Ahn et al 2006; Souchet et al 2014). However, the Ts1Rhr that is trisomic for *Dyrk1a* plus ~30 additional genes shows normal MWM performance (Olsen et al 2007). Thus, the effects of trisomy and elevated expression of *Dyrk1A* alone are modulated by the increased copy number of additional genes (or genetic background). This observation indicates that the consequences of trisomy of *DYRK1A* in DS may be different again from those in the single gene transgenics and partial trisomy mice. These differences could also influence responses to drugs that inhibit *DYRK1A* activity (Becker et al 2015).

(ii) Effects of trisomy of *Dyrk1a* and *Kcjn6* are not additive

The Dp16 mouse is impaired in the T-maze and context fear conditioning (CFC), and shows decreased long term depression (LTP) (Li et al 2007). Reducing *Dyrk1a* to two copies by crossing the Dp16 with a *Dyrk1a* knockout partially rescued these abnormalities, improving performance, but not to levels of euploid controls (performance in the MWM was not tested) (Jiang et al 2015). However, simultaneously reducing to two copies *Dyrk1a* and the adjacent potassium voltage-gated channel subunit gene, *Kcjn6*, failed to produce improvement in

these same features. Current knowledge provides no mechanistic understanding of how normalization of *Kcnj6* levels in the context of the Dp16 trisomic segment abrogates the beneficial effects of normalization of *Dyrk1a* levels. One interpretation is that trisomy of *Kcnj6* provides some protection from more severe deficits due to trisomy of some subset of Dp16 trisomic genes. This protection must also be context specific because a mouse overexpressing *Kcnj6* alone also displays deficits in CFC (Cooper et al 2012). An alternative explanation is suggested by inspection of the genomic sequence annotation: two lncRNAs are present, one antisense to *Kcnj6* and the other located between *Dyrk1a* and *Kcnj6*. It is possible that these genes have relevant unanticipated functions in regulation of expression or activity of genes elsewhere in the Dp16 trisomic segment. These results, extended by Vidal et al 2012, also emphasize the potential pitfalls of proposing individual Hsa21 genes or gene products (Jiang et al 2015) as targets for therapeutics to rescue cognitive deficits.

(iii) Trisomy of the Mmu17 segment influences effects of Mmu16 trisomy

The Ms1Rhr mouse carries a deletion created using chromosomal engineering and is monosomic for the 29 genes (*Cbr1-Fam3b*) trisomic in the Ts1Rhr (Olson et al 2004). Crossing the Dp16 with Ms1Rhr reduced to disomy these 29 genes and resulted in rescue of performance in the MWM, improvement in performance in the T-maze and CFC, and partial amelioration of LTP deficits (Zhang et al 2014). However, in the presence of trisomy for the Mmu17 region (i.e. by first crossing Dp16 mice with the Dp17), these phenotypic improvements were not seen (Zhang et al. 2014). This is in spite of the fact that the Dp17 themselves show no deficits in the same tasks and also show enhanced LTP (Yu et al 2010b). Functions of Dp17 genes, therefore, must be influencing those of genes trisomic within the Dp16, either exacerbating effects of those that cause the deficits or inhibiting the ameliorating effects of deletion of the *Cbr1-Fam3b* segment.

(iv) Interactions among Mmu17 genes

The Ts1Yah mice, that are trisomic for 12 of the 19 Hsa21 orthologs trisomic in the Mmu17 region, show enhanced performance in the MWM and enhanced LTP (Pereira et al 2009). An attractive candidate in the Mmu17 region is the trefoil factor 3 (TFF3) gene; it encodes a neuropeptide that enhances learning and memory (Shi et al. 2012). Interpretation is complicated, however, because in contrast to performance in the MWM, the Ts1Yah are impaired in both NOR and the Y-maze (Pereira et al. 2009). Furthermore, the Dp17, that are trisomic for the entire Mmu17 syntenic region, show neither enhanced MWM learning nor impaired NOR and Y-maze performance (Yu et al 2010b). Thus, one or more of the 7 genes uniquely trisomic in the Dp17 must be providing protection from both the positive and the deleterious effects of genes trisomic in the Ts1Yah. Both the Ts1Yah and the Dp17 are also trisomic for the phosphodiesterase 9a (PDE9A) gene, a member of the family of 3'-5' cyclic nucleotide phosphodiesterase, PDEs. PDEs regulate intracellular signaling through degradation of cAMP and cGMP and play roles in mood and learning and memory. Inhibition of PDEs is suggested to be protective (Xu et al. 2011) and specific inhibition of PDE9A activity produces enhanced LTP (Hutson et al. 2011; Kroker et al. 2012). These observations are inconsistent with the phenotypes of the Ts1Yah and the Dp17. Current information on the additional seven genes trisomic in the Dp17 but not in the Ts1Yah does not suggest explanations for the phenotypic differences between the two trisomies.

(v) Interactions between Mmu17 and Mmu10 genes

When Dp17 mice were crossed with Dp10, the double trisomics were reported to show normal LTP (Zhang et al 2014). Therefore, trisomy of the Mmu10 region modulated the elevated LTP observed in the Dp17 alone.

(vi) Interactions among Dp10 genes

In vivo and in vitro studies with individual genes from the Mmu10 orthologous region have revealed a number of functions relevant to both brain development and brain function (Table 4). Male Dp10 mice, however, have been reported to show no deficits in the MWM or CFC nor abnormalities in LTP at 2–4 months of age (Yu et al. 2010b). The beneficial or detrimental effects of trisomy of genes within the Mmu10 region thus remain to be determined.

The data in Tables 3 and 4 were selected to illustrate the challenges in determining gene-phenotype correlations in trisomy. They emphasize the inherent limitations of focusing on individual Hsa21 genes as having unique importance to the DS phenotype or as being effective drug targets. The phenotypic consequences of trisomy of Hsa21 genes are influenced by unknown and unpredictable functional interactions among trisomic genes. That such interactions exist is not a new concept (Olson et al. 2004) but is often ignored. When clinical trials are based on results of preclinical evaluations carried out in a single, partially trisomic, mouse model, effects of genomic context may result in poor or non-significant outcomes.

Preclinical evaluations in the Ts65Dn and clinical trials for cognition in DS

Currently, the only mouse model used in preclinical evaluations of drugs for cognitive enhancement in DS is the Ts65Dn (reviewed in Gardiner 2014). Based on these evaluations, clinical trials have been conducted using antioxidants, memantine (a drug approved for treatment of Alzheimer's Disease), and an extract of green tea (Lott et al 2011; Hanney et al 2012; Boada et al 2012; De la Torre et al 2014). Additional trials are in progress or planned, and now include discussions of prenatal treatments (Kuehn et al. 2016; Bartesaghi et al 2015). Clinical trials so far have produced at best very marginally positive results. Trials in DS are not unique in their limited efficacy. Across therapeutic areas, it is estimated that, of clinical trials following from preclinical evidence in mice, >80% fail (Perrin 2014). Proponents of DS trials argue that larger trials, longer trials, trials using younger participants (or fetuses), or trials supplemented with cognitive training are necessary to produce significant improvements in the functioning of a brain that has been perturbed throughout development. These are reasonable proposals but it is also necessary to optimize preclinical evaluations. Issues to be considered include inadequate sample size, use only of male mice, lack of genetic diversity in the models, failures to randomize assignment to experimental groups, and failures to blind the experimenter to genotype and treatment (Perrin 2014; Macleod 2014; McGonigle and Ruggeri 2014), and, in the realm of cognition, use of too limited a number and range of learning/memory assessments. While improvements in these areas are important, for cognition in DS, it is also necessary to address the fact that the Ts65Dn does not represent the genetic perturbation that is DS.

Phenotypic features of the Ts65Dn mimic many of those seen in DS, e.g. in behavior, LM, and brain region and cellular abnormalities, and some (e.g. cerebellar cellular abnormalities; Baxter et al. 2000) even served to predict previously undocumented details of the DS phenotype (reviewed in Rueda et al 2012). Thus, the Ts65Dn has been of inestimable value to DS research. It cannot be concluded, however, as many implicitly do in limiting their studies to only the Ts65Dn, that the trisomic genes causing a phenotypic feature in the Ts65Dn, and only those genes, cause the same feature in DS. To do so is a failure of disciplined reasoning and flies in the face of evidence in Tables 3 and 4. It makes two tacit, scientifically unsupportable, assumptions: (i) Hsa21 genes not trisomic in the Ts65Dn make no contribution to the DS phenotype and do not influence drug responses, and conversely (ii) genes trisomic in the Ts65Dn that are not orthologs of Hsa21 genes, i.e. the Hsa6 orthologs, do not contribute to the Ts65Dn phenotype or to drug responses. Too much is known about these respective Hsa21 and Hsa6 genes to dismiss them.

i) Hsa21 orthologs not trisomic in the Ts65Dn

The collective evidence is very strong that Hsa21 orthologs not trisomic in the Ts65Dn influence the DS phenotype. For example, among the Hsa21 Mmu16 orthologs not trisomic are HSPA13, the Hsa21-encoded heat shock protein functioning in the ubiquitin pathway, that regulates intracellular pH in neurons (Bae et al. 2013); NRIP1, the nuclear hormone interacting protein, that regulates transcriptional activity of glucocorticoid, estrogen and other receptors, and that participates in muscle metabolism and adipocyte function (White et al. 2008); and NCAM2, a neural cell adhesion molecule, that functions in axon and dendrite compartmentalization (Winther et al. 2012) and that results in neurodevelopmental disorders when regionally deleted (Petit et al. 2015). Other non-trisomic Hsa21 Mmu16 orthologs are the putative tumor suppressor gene, BTG3 (Winkler 2010) and the lncRNA/microRNA host gene Mir99ahg. Some of the Mmu17 and Mmu10 Hsa21 orthologs with potential contributions to DS features were discussed above (Table 4; see Block et al 2014, 2015 for further details).

ii) Non-Hsa21 orthologs trisomic in the Ts65Dn

Consideration of the functional features of the 35 Hsa6 protein coding genes trisomic in the Ts65Dn is important because they are overexpressed in the Ts65Dn (Duchon et al. 2011). For example, the Sorting Nexin 9 (SNX9) gene functions in clathrin-mediated endocytosis; overexpression in cultured hippocampal neurons results in defects in synaptic vesicle endocytosis (Shin et al. 2007). SNX9, along with clathrin, also has a role in mitosis, based on observations that mutations in SNX9 cause abnormal chromosomal alignment and segregation (Ma et al. 2013). Furthermore, the *Drosophila* ortholog of SNX9 is found in a complex with the ortholog of the Hsa21 protein DSCAM, and it interacts with components of the actin polymerization machinery and with clathrin adaptor proteins, similar to observations in hippocampal neurons (Worby et al. 2001). A second example involves the dynein light chain genes (*Dynlt*), five of which are trisomic in the Ts65Dn. Cytosolic dynein is a molecular motor participating in retrograde axonal transport of signaling endosomes containing NGF, BDNF and their receptors, TRKA and TRKB (Zhou et al. 2012). The dynein complex is composed of multiple proteins classed as heavy, intermediate, intermediate-light and light chains. Overexpression of light chains alters the composition of

signaling endosomes and decreases downstream signaling through MAPK (Duguay et al. 2011). Both SNX9 and Dnlyta-e proteins are worth considering for contributions to abnormalities in endosomes and NGF retrograde transport observed in the Ts65Dn (Salehi et al. 2006).

Paralogs of several Hsa21/Mmu16 genes also map to the Ts65Dn Mmu17 segment. These include: Synj2, a paralog of the Hsa21 phosphatidylinositol phosphatase, SYNJ1 that is known to contribute to LM deficits; and TIAM2, a paralog of the Hsa21 guanine nucleotide exchange factor, TIAM1, that is involved in neurite outgrowth. Scaf8 is a paralog of the Hsa21 serine-arginine rich splicing factor Scaf15, and claudin 20 (CLDN20) is a paralog of the Hsa21 tight junction component genes CLDN8, 14 and 17. Tcp10a and Tcp10b are paralogs of the human-specific transcription factor T-complex 10-like, Tcp10L, that is involved in spermatogenesis (Yu et al. 2005). The Pde10a gene is paralog of the Hsa21 gene Pde9a. Pairs of paralogous genes may differ in regulation and functional properties, but may also overlap in some features. Therefore, the addition of such genes to a trisomy may have phenotypic consequences.

It is unlikely that every DS relevant phenotypic feature of the Ts65Dn is significantly impacted by the non-Hsa21 orthologous trisomic genes. Indeed, the comparative analysis of several DS models trisomic for segments of Mmu16 robustly supports the Mmu16 origin of the Ts65Dn craniofacial abnormalities relevant to DS (Starbuck et al 2014). However, as illustrated in Tables 3 and 4, the same conclusion is not currently possible for other phenotypic features and it is unscientific to fail to consider that non-Hsa21 orthologs influence either phenotypic features of the Ts65Dn or responses to drug treatments.

iii) Additional limitations of the Ts65Dn

Another concern also rarely discussed is that male Ts65Dn are largely infertile and most commonly colonies are maintained by breeding trisomic females to euploid males (Moore et al 2010). This means that Ts65Dn mice used in preclinical studies derive from gestation in trisomic dams, with unknown consequences for fetal development. This also means that rarely are female Ts65Dn used in such studies (Gardiner 2014), leaving undetected potential sex differences.

Alternatives to the Ts65Dn, and compromises, for preclinical drug evaluations

Mice trisomic for all Hsa21 orthologs can be obtained by crossing the Dp10 with the Dp17, and using the double trisomic offspring to cross with the Dp16 to obtain a “triple trisomy”. This breeding is time consuming and expensive. Two generations of breeding are required to obtain each mouse and final yields are much lower than expected. While a cross between Dp10 and Dp17 produces the expected $\frac{1}{4}$ double trisomics, when these are crossed with the Dp16, there are losses in utero due to heart defects, as seen in the Ts65Dn and the Dp16 alone, and further decreases are caused by the deleterious effects of the complete trisomy (also seen in DS fetuses). For preclinical evaluations with behavior testing or other analyses where it is desirable to have a dozen or more age- and sex-matched individuals, with and

without drug treatment, generating sufficient numbers becomes impractical for most researchers, and certainly for extensive screening of potential drug treatments. This is likely reflected in the appearance of only two publications using the triple trisomic mice (Yu et al 2010a; Belichenko et al 2015), and none with preclinical evaluations.

It is inarguable that the Dp16 mouse, although also itself not ideal, is a better genetic model of DS than the Ts65Dn: it is trisomic for the entire Mmu16 syntenic region and is not trisomic for any non-Hsa21 orthologs, and both males and females are fertile, thus eliminating the need to breed with trisomic females. It remains to be more fully determined how different and similar the Dp16 vs. the Ts65Dn phenotypes are (Starbuck et al 2014; Goodliffe et al 2016). Where they differ from the Ts65Dn, the potential reasons include the following: (i) the feature is modified or ameliorated by the additional Mmu16 Hsa21 orthologs trisomic in the Dp16 but not in the Ts65Dn; (ii) the feature was caused in whole or in part by genes in the Ts65Dn mapping to the centromeric, Hsa6 syntenic region of Mmu17; or (iii) the feature is affected by differences in genetic backgrounds or differences in expression of trisomic genes present in an internal duplication vs. on a separate chromosome. These causes are not mutually exclusive and they also are not straightforward to determine. It is also argued that, because the majority of DS is due to an extra freely segregating Hsa21, the Ts65Dn is preferable to the Dp16 because the latter has an internal duplication and lacks an extra chromosome. While the Ts65Dn may be of interest for studies of chromosomal segregation, it is difficult to argue that for DS studies this feature outweighs the additional genetic limitations.

Drugs that have been successful in rescuing learning/memory in the Ts65Dn are diverse in their known targets and mechanisms of action (reviewed in Gardiner 2014). There is no understanding of how these drugs directly or indirectly influence the overexpression or elevated activities of trisomic genes to result in these rescues. As a result, it cannot be predicted that drug responses in the Ts65Dn, or the Dp16, will be replicated to any significant effect in full trisomy. It is, therefore, necessary to include trisomy of Mmu17 and Mmu10 genes in preclinical evaluations. Analysis of the individual, single trisomics, Dp17 and Dp10 is uncomplicated and, at least, would ensure that all Hsa21 orthologs are interrogated. Analysis of double trisomics, i.e. offspring obtained by crossing two of the Dp16, Dp17 and Dp10, may be informative compromises for full trisomy. For example, based on data from Zhang et al (2014), prior to clinical trials, it would be useful to know if a drug that rescued deficits in the Ts65Dn, not only rescued the same deficits in the Dp16, but also in the double trisomic Dp16/Dp17. The Dp16/Dp10 double trisomic would determine how Dp10 genes affect responses to anti-oxidants (S100B) and melatonin and memantine (TRPM2) (Table 4) that demonstrated efficacy in the Ts65Dn. Admittedly, it is expensive in time and resources to replicate in additional mouse models all the studies reported for the Ts65Dn, but it is also expensive to have clinical trials fail.

Conclusions

DS had long been considered by many to be too complicated a genetic condition to be amenable to productive study and certainly too complicated for successful drug therapy. Contrary to that pessimism, the last decade has brought the development of several mouse

models and the unprecedented demonstration that learning and memory deficits can be rescued in adult Ts65Dn mice with a large and diverse array of drugs. Effective therapy for cognition in DS now seems an attainable goal. The enthusiasm for the goal however needs to be tempered with critical evaluations of the limits of current knowledge and resources, and with rigorous consideration of design and interpretation of preclinical results obtained with partially trisomic mouse models.

For a small number of Hsa21 protein coding genes, there is considerable functional and overexpression phenotypic information. For others, there are functional data from studies not directed at DS. For the large majority, however, there is little beyond sequence similarities. The cohort of lncRNA genes remain largely unexplored, but not without potential importance. These facts mean that predicting (or excluding) specific genes, particularly those in the Mmu17 and Mmu10 regions, as the causes of any DS phenotypic feature or as the mediators or modulators of a drug response is currently not possible.

It is being strongly argued in the recent literature that the first criterion for evaluating the usefulness of a mouse model of human disease must be its genetic validity, not mere phenotypic similarity (Perrin 2014; McGonigle and Ruggeri 2014; Macleod 2014). For DS, genetic validity is dictated by the presence and absence of trisomy of specific genes. The extent of the genetic limitations of the Ts65Dn have been made clear: 25% of trisomic genes are not Hsa21 orthologs and 45% of Hsa21 orthologs are not trisomic. As uniquely valuable as the Ts65Dn has been, it was never justifiable for use in preclinical evaluations of drug treatments. Its continued use needs to be driven by more than just history and popularity.

There is no simple, complete trisomy mouse model of DS. This does not mean that mouse models are not useful. Rather it means that multiple models, with differing complements of trisomic genes, need to be investigated. Understanding the value and the limitations of each of the partial trisomy models requires thoughtful consideration of the number and nature, not only of the Hsa21 orthologs that are trisomic, and therefore presumably driving the phenotype of the model, but also of the Hsa21 orthologs that are not trisomic. Their functions and possible interactions with trisomic genes and in drug responses that would differentially occur in people with DS and full trisomy Hsa21 need to be determined. Only then can phenotypic features, and their rescue by drug treatments, begin to be useful predictors for efficacy in clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Ahn KJ, Jeong HK, Choi HS, Ryoo SR, Kim YJ, Goo JS, Choi SY, Han JS, Ha I, Song WJ. DYRK1A BAC transgenic mice show altered synaptic plasticity with learning and memory defects. *Neurobiol Dis.* 2006; 22:463–472. [PubMed: 16455265]
- Al Ahmad A, Lee B, Stack J, Parham C, Campbell J, Clarke D, Fertala A, Bix GJ. Endostatin binds nerve growth factor and thereby inhibits neurite outgrowth and neuronal migration in-vitro. *Brain Res.* 2010; 1360:28–39. [PubMed: 20846515]
- Alim I, Teves L, Li R, Mori Y, Tymianski M. Modulation of NMDAR subunit expression by TRPM2 channels regulates neuronal vulnerability to ischemic cell death. *J Neurosci.* 2013; 33:17264–17277. [PubMed: 24174660]
- Andrews SJ, Rothnagel JA. Emerging evidence for functional peptides encoded by short open reading frames. *Nat Rev Genet.* 2014; 15:193–204. [PubMed: 24514441]
- Antonarakis SE, Lyle R, Dermitzakis ET, Reymond A, Deutsch S. Chromosome 21 and down syndrome: from genomics to pathophysiology. *Nat Rev Genet.* 2004; 5:725–38. [PubMed: 15510164]
- Bae JS, Koo NY, Namkoong E, Davies AJ, Choi SK, Shin Y, Jin M, Hwang SM, Mikoshiba K, Park K. Chaperone stress 70 protein (STCH) binds and regulates two acid/base transporters NBCe1-B and NHE1. *J Biol Chem.* 2013; 288:6295–6305. [PubMed: 23303189]
- Ballarino M, Cazzella V, D'Andrea D, Grassi L, Bisceglie L, Cipriano A, Santini T, Pinnarò C, Morlando M, Tramontano A, Bozzoni I. Novel long noncoding RNAs (lncRNAs) in myogenesis: a miR-31 overlapping lncRNA transcript controls myoblast differentiation. *Mol Cell Biol.* 2015; 35:728–36. [PubMed: 25512605]
- Bartasaghi R, Haydar TF, Delabar JM, Dierssen M, Martínez-Cué C, Bianchi DW. New Perspectives for the Rescue of Cognitive Disability in Down Syndrome. *J Neurosci.* 2015; 35:13843–52. [PubMed: 26468184]
- Baxter LL, Moran TH, Richtsmeier JT, Troncoso J, Reeves RH. Discovery and genetic localization of Down syndrome cerebellar phenotypes using the Ts65Dn mouse. *Hum Mol Genet.* 2000; 9:195–202. [PubMed: 10607830]
- Becker W, Soppa U, Tejedor FJ. DYRK1A: a potential drug target for multiple Down syndrome neuropathologies. *CNS Neurol Disord Drug Targets.* 2014; 13:26–33. [PubMed: 24152332]
- Begley CG, Ellis LM. Drug development: Raise standards for preclinical cancer research. *Nature.* 2012; 483:531–533. [PubMed: 22460880]
- Bergmann JH, Spector DL. Long non-coding RNAs: modulators of nuclear structure and function. *Curr Opin Cell Biol.* 2014; 26:10–8. [PubMed: 24529241]
- Belichenko PV, Kleschevnikov AM, Becker A, Wagner GE, Lysenko LV, Yu YE, Mobley WC. Down Syndrome Cognitive Phenotypes Modeled in Mice Trisomic for All HSA 21 Homologues. *PLoS One.* 2015; 10:e0134861. [PubMed: 26230397]
- Bera TK, Zimonjic DB, Popescu NC, Sathyanarayana BK, Kumar V, Lee B, Pastan I. POTE, a highly homologous gene family located on numerous chromosomes and expressed in prostate, ovary, testis, placenta, and prostate cancer. *Proc Natl Acad Sci U S A.* 2002; 99:16975–16980.
- Bialowas-McGoey LA, Lesicka A, Whitaker-Azmitia PM. Vitamin E increases S100B-mediated microglial activation in an S100B-overexpressing mouse model of pathological aging. *Glia.* 2008; 56:1780–1790. [PubMed: 18649404]
- Boada R, Hutaff-Lee C, Schrader A, Weitzenkamp D, Benke TA, Goldson EJ, Costa AC. Antagonism of NMDA receptors as a potential treatment for Down syndrome: a pilot randomized controlled trial. *Transl Psychiatry.* 2012; 2:e141. [PubMed: 22806212]
- Bofill-De Ros X, Santos M, Vila-Casadesús M, Villanueva E, Andreu N, Dierssen M, Fillat C. Genome-wide miR-155 and miR-802 target gene identification in the hippocampus of Ts65Dn Down syndrome mouse model by miRNA sponges. *BMC Genomics.* 2015; 16:907. [PubMed: 26546125]
- Block A, Dhanasekaran AR, Ahmed MD, Gardiner KJ. Abnormal protein profiles in hippocampus of mouse models of Down syndrome: similarities with Alzheimer's Disease. *J Alzheimer's Disease and Parkinsonism.* 2014; 4:138–149.

- Block A, Ahmed MM, Dhanasekaran AR, Tong S, Gardiner KJ. Sex differences in protein expression in the mouse brain and their perturbations in a model of Down syndrome. *Biol Sex Differ*. 2015; 6:24. [PubMed: 26557979]
- Blythe SA, Cha SW, Tadjuidje E, Heasman J, Klein PS. beta-Catenin primes organizer gene expression by recruiting a histone H3 arginine 8 methyltransferase, Prmt2. *Dev Cell*. 2010; 19:220–31. [PubMed: 20708585]
- Brault V, Duchon A, Romestaing C, Sahun I, Pothion S, Karout M, Borel C, Dembele D, Bizot JC, Messaddeq N, Sharp AJ, Roussel D, Antonarakis SE, Dierssen M, Héroult Y. Opposite phenotypes of muscle strength and locomotor function in mouse models of partial trisomy and monosomy 21 for the proximal Hspa13-App region. *PLoS Genet*. 2015; 11:e1005062. [PubMed: 25803843]
- Bruford EA, Lane L, Harrow J. Devising a Consensus Framework for Validation of Novel Human Coding Loci. *J Proteome Res*. 2015; 14:4945–8. [PubMed: 26367542]
- Canfield MA, Honein MA, Yuskiv N, Xing J, Mai CT, Collins JS, Devine O, Petrini J, Ramadhani TA, Hobbs CA, Kirby RS. National estimates and race/ethnic-specific variation of selected birth defects in the United States, 1999–2001. *Birth Defects Res A Clin Mol Teratol*. 2006; 76:747–756. [PubMed: 17051527]
- Capone GT. Down syndrome: advances in molecular biology and the neurosciences. *J Dev Behav Pediatr*. 2001; 22:40–59. [PubMed: 11265922]
- Celik O, Naziro lu M. Melatonin modulates apoptosis and TRPM2 channels in transfected cells activated by oxidative stress. *Physiol Behav*. 2012; 107:458–65. [PubMed: 23041488]
- Chapman RS, Hesketh LJ. Behavioral phenotype of individuals with Down syndrome. *Ment Retard Dev Disabil Res Rev*. 2000; 6:84–95. [PubMed: 10899801]
- Choong XY, Tosh JL, Pulford LJ, Fisher EM. Dissecting Alzheimer disease in Down syndrome using mouse models. *Front Behav Neurosci*. 2015; 9:268. [PubMed: 26528151]
- Choucair-Jaafar N, Laporte V, Levy R, Poindron P, Lombard Y, Gies JP. Complement receptor 3 (CD11b/CD18) is implicated in the elimination of β -amyloid peptides. *Fundam Clin Pharmacol*. 2011; 25:115–22. [PubMed: 20199584]
- Cooper A, Grigoryan G, Guy-David L, Tsoory MM, Chen A, Reuveny E. Trisomy of the G protein-coupled K⁺ channel gene, *Kcnj6*, affects reward mechanisms, cognitive functions, and synaptic plasticity in mice. *Proc Natl Acad Sci U S A*. 2012; 109:2642–7. [PubMed: 22308328]
- Davisson MT, Schmidt C, Akeson E. Segmental trisomy of murine chromosome 16: a new model system for studying Down syndrome. *Prog Clin Biol Res*. 1990; 360:263–280. [PubMed: 2147289]
- Davisson MT, Schmidt C, Reeves RH, Irving NG, Akeson EC, Harris BS, Bronson RT. Segmental trisomy as a mouse model for Down syndrome. *Prog Clin Biol Res*. 1993; 384:117–133. [PubMed: 8115398]
- Davisson MT, Bechtel LJ, Akeson EC, Fortna A, Slavov D, Gardiner K. Evolutionary breakpoints on human chromosome 21. *Genomics*. 2001; 78:99–106. [PubMed: 11707078]
- de Hoon M, Shin JW, Carninci P. Paradigm shifts in genomics through the FANTOM projects. *Mamm Genome*. 2015; 26:391–402. [PubMed: 26253466]
- Deiningner MH, Fimmen BA, Thal DR, Schluesener HJ, Meyermann R. Aberrant neuronal and paracellular deposition of endostatin in brains of patients with Alzheimer's disease. *J Neurosci*. 2002; 22:10621–10626. [PubMed: 12486154]
- Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhatter R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigó R. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. *Genome Res*. 2012; 22:1775–1789. [PubMed: 22955988]
- De la Torre R, De Sola S, Pons M, Duchon A, de Lagran MM, Farré M, Fitó M, Benejam B, Langohr K, Rodriguez J, Pujadas M, Bizot JC, Cuenca A, Janel N, Catuara S, Covas MI, Blehaut H, Héroult Y, Delabar JM, Dierssen M. Epigallocatechin-3-gallate, a DYRK1A inhibitor, rescues cognitive deficits in Down syndrome mouse models and in humans. *Mol Nutr Food Res*. 2014; 58:278–88. [PubMed: 24039182]

- Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, Tubaro C, Giambanco I. S100B's double life: intracellular regulator and extracellular signal. *Biochim Biophys Acta*. 2009; 1793:1008–1022. [PubMed: 19110011]
- Duchon A, Raveau M, Chevalier C, Nalesso V, AJ S, Hérault Y. Identification of the translocation breakpoints in the Ts65Dn and Ts1Cje mouse lines: relevance for modeling Down syndrome. *Mamm Genome*. 2011; 22:674–684. [PubMed: 21953411]
- Duguay D, Bélanger-Nelson E, Mongrain V, Beben A, Khatchadourian A, Cermakian N. Dynein light chain Tctex-type 1 modulates orexin signaling through its interaction with orexin 1 receptor. *PLoS One*. 2011; 6:e26430. [PubMed: 22028875]
- Emmrich S, Streltsov A, Schmidt F, Thangapandi VR, Reinhardt D, Klusmann JH. LincRNAs MONC and MIR100HG act as oncogenes in acute megakaryoblastic leukemia. *Mol Cancer*. 2014; 13:171. [PubMed: 25027842]
- Faraoni I, Antonetti FR, Cardone J, Bonmassar E. miR-155 gene: a typical multifunctional microRNA. *Biochim Biophys Acta*. 2009; 1792:497–505. [PubMed: 19268705]
- Feliciano A, Castellvi J, Artero-Castro A, Leal JA, Romagosa C, Hernández-Losa J, Peg V, Fabra A, Vidal F, Kondoh H, Ramón Y, Cajal S, Lleonart ME. miR-125b acts as a tumor suppressor in breast tumorigenesis via its novel direct targets ENPEP, CK2- α , CCNJ, and MEGF9. *PLoS One*. 2013; 8:e76247. [PubMed: 24098452]
- Flicek P, Amode MR, Barrell D, Beal K, Billis K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fitzgerald S, Gil L, Girón CG, Gordon L, Hourlier T, Hunt S, Johnson N, Juettemann T, Kähäri AK, Keenan S, Kulesha E, Martin FJ, Maurel T, McLaren WM, Murphy DN, Nag R, Overduin B, Pignatelli M, Pritchard B, Pritchard E, Riat HS, Ruffier M, Sheppard D, Taylor K, Thormann A, Trevanion SJ, Vullo A, Wilder SP, Wilson M, Zadissa A, Aken BL, Birney E, Cunningham F, Harrow J, Herrero J, Hubbard TJ, Kinsella R, Muffato M, Parker A, Spudich G, Yates A, Zerbino DR, Searle SM. Ensembl 2014. *Nucleic Acids Res*. 2014; 42:D749–D755. [PubMed: 24316576]
- Flynn RA, Chang HY. Long noncoding RNAs in cell-fate programming and reprogramming. *Cell Stem Cell*. 2014; 14:752–761. [PubMed: 24905165]
- García-Cerro S, Martínez P, Vidal V, Corrales A, Flórez J, Vidal R, Rueda N, Arbonés ML, Martínez-Cué C. Overexpression of Dyrk1A is implicated in several cognitive, electrophysiological and neuromorphological alterations found in a mouse model of Down syndrome. *PLoS One*. 2014; 9:e106572. [PubMed: 25188425]
- Gardiner K, Slavov D, Bechtel L, Davisson M. Annotation of human chromosome 21 for relevance to Down syndrome: gene structure and expression analysis. *Genomics*. 2002; 79:833–43. [PubMed: 12036298]
- Gardiner KJ. Pharmacological approaches to improving cognitive function in Down syndrome: current status and considerations. *Drug Des Devel Ther*. 2014; 17:103–125.
- Goff LA, Groff AF, Sauvageau M, Traves-Gibson Z, Sanchez-Gomez DB, Morse M, Martin RD, Elcavage LE, Liapis SC, Gonzalez-Celeiro M, Plana O, Li E, Gerhardinger C, Tomassy GS, Arlotta P, Rinn J. Spatiotemporal expression and transcriptional perturbations by long noncoding RNAs in the mouse brain. *Proc Natl Acad Sci U S A*. 2015; 112:6855–6862. [PubMed: 26034286]
- Goodliffe JW, Olmos-Serrano JL, Aziz NM, Pennings JL, Guedj F, Bianchi DW, Haydar TF. Absence of Prenatal Forebrain Defects in the Dp(16)1Yey/+ Mouse Model of Down Syndrome. *J Neurosci*. 2016; 36:2926–44. [PubMed: 26961948]
- Gray KA, Yates B, Seal RL, Wright MW, Bruford EA. Genenames.org: the HGNC resources in 2015. *Nucleic Acids Res*. 2015; 43:D1079–85. [PubMed: 25361968]
- Gribble SM, Wiseman FK, Clayton S, Prigmore E, Langley E, Yang F, Maguire S, Fu B, Rajan D, Sheppard O, Scott C, Hauser H, Stephens PJ, Stebbings LA, Ng BL, Fitzgerald T, Quail MA, Banerjee R, Rothkamm K, Tybulewicz VL, Fisher EM, Carter NP. 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*. 2013; 8:e60482. [PubMed: 23596509]
- Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S, Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature*. 2010; 464:1071–6. [PubMed: 20393566]

- Hanney M, Prasher V, Williams N, Jones EL, Aarsland D, Corbett A, Lawrence D, Yu LM, Tyrer S, Francis PT, Johnson T, Bullock R, Ballard C, MEADOWS trial researchers. Memantine for dementia in adults older than 40 years with Down's syndrome (MEADOWS): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2012; 379:528–536. [PubMed: 22236802]
- Harrow J, Frankish A, Gonzalez JM, Tapanari E, Diekhans M, Kokocinski F, Aken BL, Barrell D, Zadissa A, Searle S, Barnes I, Bignell A, Boychenko V, Hunt T, Kay M, Mukherjee G, Rajan J, Despacio-Reyes G, Saunders G, Steward C, Harte R, Lin M, Howald C, Tanzer A, Derrien T, Chrast J, Walters N, Balasubramanian S, Pei B, Tress M, Rodriguez JM, Ezkurdia I, van Baren J, Brent M, Haussler D, Kellis M, Valencia A, Reymond A, Gerstein M, Guigó R, Hubbard TJ. GENCODE: the reference human genome annotation for The ENCODE Project. *Genome Res*. 2012; 22:1760–1774. [PubMed: 22955987]
- Harrow JL, Steward CA, Frankish A, Gilbert JG, Gonzalez JM, Loveland JE, Mudge J, Sheppard D, Thomas M, Trevanion S, Wilming LG. The Vertebrate Genome Annotation browser 10 years on. *Nucleic Acids Res*. 2014; 42:D771–779. [PubMed: 24316575]
- Hattori M, Fujiyama A, Taylor TD, Watanabe H, Yada T, Park HS, Toyoda A, Ishii K, Totoki Y, Choi DK, Groner Y, Soeda E, Ohki M, Takagi T, Sakaki Y, Taudien S, Blechschmidt K, Polley A, Menzel U, Delabar J, Kumpf K, Lehmann R, Patterson D, Reichwald K, Rump A, Schillhabel M, Schudy A, Zimmermann W, Rosenthal A, Kudoh J, Schibuya K, Kawasaki K, Asakawa S, Shintani A, Sasaki T, Nagamine K, Mitsuyama S, Antonarakis SE, Minoshima S, Shimizu N, Nordsiek G, Hornischer K, Brant P, Scharfe M, Schon O, Desario A, Reichelt J, Kauer G, Blocker H, Ramser J, Beck A, Klages S, Hennig S, Riesselmann L, Dagand E, Haaf T, Wehrmeyer S, Borzym K, Gardiner K, Nizetic D, Francis F, Lehrach H, Reinhardt R, Yaspo ML, Chromosome 21 mapping and sequencing consortium. The DNA sequence of human chromosome 21. *Nature*. 2000; 405:311–319. [PubMed: 10830953]
- Head E, Lott IT, Wilcock DM, Lemere CA. Aging in Down Syndrome and the Development of Alzheimer's Disease Neuropathology. *Curr Alzheimer Res*. 2016; 13:18–29. [PubMed: 26651341]
- Hilton JF, Christensen KE, Watkins D, Raby BA, Renaud Y, de la Luna S, Estivill X, MacKenzie RE, Hudson TJ, Rosenblatt DS. The molecular basis of glutamate formiminotransferase deficiency. *Hum Mutat*. 2003; 22:67–73. [PubMed: 12815595]
- Horsch M, Seeburg PH, Adler T, Aguilar-Pimentel JA, Becker L, Calzada-Wack J, Garrett L, Götz A, Hans W, Higuchi M, Hölter SM, Naton B, Prehn C, Puk O, Rác I, Rathkolb B, Rozman J, Schrewe A, Adamski J, Busch DH, Esposito I, Graw J, Ivandic B, Klingenspor M, Klopstock T, Mempel M, Ollert M, Schulz H, Wolf E, Wurst W, Zimmer A, Gailus-Durner V, Fuchs H, de Angelis MH, Beckers J. Requirement of the RNA-editing enzyme ADAR2 for normal physiology in mice. *J Biol Chem*. 2011; 286:18614–18622. [PubMed: 21467037]
- Hüttenhofer A, Schattner P, Polacek N. Non-coding RNAs: hope or hype? *Trends Genet*. 2005; 21:289–97. [PubMed: 15851066]
- Hutson PH, Finger EN, Magliaro BC, Smith SM, Converso A, Sanderson PE, Mullins D, Hyde LA, Eschle BK, Turnbull Z, Sloan H, Guzzi M, Zhang X, Wang A, Rindgen D, Mazzola R, Vivian JA, Eddins D, Uslaner JM, Bednar R, Gambone C, Le-Mair W, Marino MJ, Sachs N, Xu G, Parmentier-Batteur S. The selective phosphodiesterase 9 (PDE9) inhibitor PF-04447943 (6-[(3S, 4S)-4-methyl-1-(pyrimidin-2-ylmethyl)pyrrolidin-3-yl]-1-(tetrahydro-2H-pyran-4-yl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one) enhances synaptic plasticity and cognitive function in rodents. *Neuropharmacology*. 2011; 61:665–676. [PubMed: 21619887]
- Irving C, Basu A, Richmond S, Burn J, Wren C. Twenty-year trends in prevalence and survival of Down syndrome. *Eur J Hum Genet*. 2008; 16:1336–1340. [PubMed: 18596694]
- Iwasaki H, Kovacic JC, Olive M, Beers JK, Yoshimoto T, Crook MF, Tonelli LH, Nabel EG. Disruption of protein arginine N-methyltransferase 2 regulates leptin signaling and produces leanness in vivo through loss of STAT3 methylation. *Circ Res*. 2010; 107:992–1001. [PubMed: 20798359]
- Jia J, Verma S, Nakayama S, Quillinan N, Grafe MR, Hurn PD, Herson PS. Sex differences in neuroprotection provided by inhibition of TRPM2 channels following experimental stroke. *J Cereb Blood Flow Metab*. 2011; 31:2160–8. [PubMed: 21587268]

- Jiang X, Liu C, Yu T, Zhang L, Meng K, Xing Z, Belichenko PV, Kleschevnikov AM, Pao A, Peresie J, Wie S, Mobley WC, Yu YE. Genetic dissection of the Down syndrome critical region. *Hum Mol Genet.* 2015; 24:6540–6551. [PubMed: 26374847]
- Kido S, Sakuragi N, Bronner MP, Sayegh R, Berger R, Patterson D, Strauss JF 3rd. D21S418E identifies a cAMP-regulated gene located on chromosome 21q22.3 that is expressed in placental syncytiotrophoblast and choriocarcinoma cells. *Genomics.* 1993; 17:256–259. [PubMed: 8406465]
- Korbel JO, Tirosh-Wagner T, Urban AE, Chen XN, Kasowski M, Dai L, Grubert F, Erdman C, Gao MC, Lange K, Sobel EM, Barlow GM, Aylsworth AS, Carpenter NJ, Clark RD, Cohen MY, Doran E, Falik-Zaccari T, Lewin SO, Lott IT, McGillivray BC, Moeschler JB, Pettenati MJ, Puschel SM, Rao KW, Shaffer LG, Shohat M, Van Riper AJ, Warburton D, Weissman S, Gerstein MB, Snyder M, Korenberg JR. The genetic architecture of Down syndrome phenotypes revealed by high-resolution analysis of human segmental trisomies. *Proc Natl Acad Sci U S A.* 2009; 106:12031–12036. [PubMed: 19597142]
- Korenberg JR, Chen XN, Schipper R, Sun Z, Gonsky R, Gerwehr S, Carpenter N, Daumer C, Dignan P, Disteche C, Graham JM Jr, Hugdins L, McGillivray B, Miyazaki K, Ogasawara N, Park JP, Pagon R, Puschel S, Sack G, Say B, Schuffenhauer S, Soukup S, Yamanaka T. Down syndrome phenotypes: the consequences of chromosomal imbalance. *Proc Natl Acad Sci U S A.* 1994; 91:4997–5001. [PubMed: 8197171]
- Kroker KS, Rast G, Giovannini R, Marti A, Dorner-Ciossek C, Rosenbrock H. Inhibition of acetylcholinesterase and phosphodiesterase-9A has differential effects on hippocampal early and late LTP. *Neuropharmacology.* 2012; 62:1964–1974. [PubMed: 22245562]
- Kuehn BM. Treating trisomies: Prenatal Down's syndrome therapies explored in mice. *Nat Med.* 2016; 22:6–7. [PubMed: 26735397]
- Lawrie CH. MicroRNAs and lymphomagenesis: a functional review. *Br J Haematol.* 2013; 160:571–581. [PubMed: 23205669]
- Liu Z, Long X, Chao C, Yan C, Wu Q, Hua S, Zhang Y, Wu A, Fang W. Knocking down CDK4 mediates the elevation of let-7c suppressing cell growth in nasopharyngeal carcinoma. *BMC Cancer.* 2014; 14:274. [PubMed: 24751144]
- Lopez-Pajares V, Qu K, Zhang J, Webster DE, Barajas BC, Sipsashvili Z, Zarnegar BJ, Boxer LD, Rios EJ, Tao S, Kretz M, Khavari PA. A lncRNA-MAF:MAFB transcription factor network regulates epidermal differentiation. *Dev Cell.* 2015; 32:693–706. [PubMed: 25805135]
- Lott IT, Doran E, Nguyen VQ, Tournay A, Head E, Gillen DL. Down syndrome and dementia: a randomized, controlled trial of antioxidant supplementation. *Am J Med Genet A.* 2011; 155A:1939–1948. [PubMed: 21739598]
- Louro R, Smirnova AS, Verjovski-Almeida S. Long intronic noncoding RNA transcription: expression noise or expression choice? *Genomics.* 2009; 93:291–8. [PubMed: 19071207]
- Li L, Chang HY. Physiological roles of long noncoding RNAs: insight from knockout mice. *Trends Cell Biol.* 2014; 24:594–602. [PubMed: 25022466]
- Li W, Notani D, Ma Q, Tanasa B, Nunez E, Chen AY, Merkurjev D, Zhang J, Ohgi K, Song X, Oh S, Kim HS, Glass CK, Rosenfeld MG. Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature.* 2013; 498:516–520. [PubMed: 23728302]
- Li Y, Wang H, Wang S, Quon D, Liu YW, Cordell B. Positive and negative regulation of APP amyloidogenesis by sumoylation. *Proc Natl Acad Sci U S A.* 2003; 100:259–64. [PubMed: 12506199]
- Li Z, Yu T, Morishima M, Pao A, LaDuca J, Conroy J, Nowak N, Matsui S, Shiraishi I, Yu YE. Duplication of the entire 22.9 Mb human chromosome 21 syntenic region on mouse chromosome 16 causes cardiovascular and gastrointestinal abnormalities. *Hum Mol Genet.* 2007; 16:1359–1366. [PubMed: 17412756]
- Lu J, Esposito G, Scuderi C, Steardo L, Delli-Bovi LC, Hecht JL, Dickinson BC, Chang CJ, Mori T, Sheen V. S100B and APP promote a gliocentric shift and impaired neurogenesis in Down syndrome neural progenitors. *PLoS One.* 2011; 6:e22126. [PubMed: 21779383]
- Ma MP, Robinson PJ, Chircop M. Sorting nexin 9 recruits clathrin heavy chain to the mitotic spindle for chromosome alignment and segregation. *PLoS One.* 2013; 8:e68387. [PubMed: 23861900]
- Macleod MR. Preclinical research: Design animal studies better. *Nature.* 2014; 510:35.

- Mattick JS. Challenging the dogma: the hidden layer of non-protein-coding RNAs in complex organisms. *Bioessays*. 2003; 25:930–9. [PubMed: 14505360]
- Mattick JS, Makunin IV. Non-coding RNA. *Hum Mol Genet*. 2006; 15(Spec No 1):R17–29. [PubMed: 16651366]
- McGonigle P. Animal models of CNS disorders. *Biochem Pharmacol*. 2014; 87:140–149. [PubMed: 23811310]
- McGonigle P, Ruggeri B. Animal models of human disease: challenges in enabling translation. *Biochem Pharmacol*. 2014; 87:162–171. [PubMed: 23954708]
- Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet*. 2009; 10:155–159. [PubMed: 19188922]
- Meyer R, Wolf SS, Obendorf M. PRMT2, a member of the protein arginine methyltransferase family, is a coactivator of the androgen receptor. *J Steroid Biochem Mol Biol*. 2007; 107:1–14. [PubMed: 17587566]
- Moore CS, Hawkins C, Franca A, Lawler A, Devenney B, Das I, Reeves RH. Increased male reproductive success in Ts65Dn “Down syndrome” mice. *Mamm Genome*. 2010; 21:543–9. [PubMed: 21110029]
- Mori T, Koyama N, Arendash GW, Horikoshi-Sakuraba Y, Tan J, Town T. Overexpression of human S100B exacerbates cerebral amyloidosis and gliosis in the Tg2576 mouse model of Alzheimer’s disease. *Glia*. 2010; 58:300–14. [PubMed: 19705461]
- Nakamura A, Hattori M, Sakaki Y. A novel gene isolated from human placenta located in Down syndrome critical region on chromosome 21. *DNA Res*. 1997; 4:321–324. [PubMed: 9455479]
- Nelson DL, Gibbs RA. Genetics. The critical region in trisomy 21. *Science*. 2004; 306:619–621. [PubMed: 15499000]
- Ng SY, Bogu GK, Soh BS, Stanton LW. The long noncoding RNA RMST interacts with SOX2 to regulate neurogenesis. *Mol Cell*. 2013; 51:349–59. [PubMed: 23932716]
- O’Doherty A, Ruf S, Mulligan C, Hildreth V, Errington ML, Cooke S, Sesay A, Modino S, Vanes L, Hernandez D, Linehan JM, Sharpe PT, Brandner S, Bliss TV, Henderson DJ, Nizetic D, Tybulewicz VL, Fisher EM. An aneuploid mouse strain carrying human chromosome 21 with Down syndrome phenotypes. *Science*. 2005; 309:2033–2037. [PubMed: 16179473]
- O’Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, Astashyn A, Badretdin A, Bao Y, Blinkova O, Brover V, Chetvernin V, Choi J, Cox E, Ermolaeva O, Farrell CM, Goldfarb T, Gupta T, Haft D, Hatcher E, Hlavina W, Joardar VS, Kodali VK, Li W, Maglott D, Masterson P, McGarvey KM, Murphy MR, O’Neill K, Pujar S, Rangwala SH, Rausch D, Riddick LD, Schoch C, Shkeda A, Storz SS, Sun H, Thibaud-Nissen F, Tolstoy I, Tully RE, Vatsan AR, Wallin C, Webb D, Wu W, Landrum MJ, Kimchi A, Tatusova T, DiCuccio M, Kitts P, Murphy TD, Pruitt KD. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res*. 2016; 44:D733–745. [PubMed: 26553804]
- Olson LE, Richtsmeier JT, Leszl J, Reeves RH. A chromosome 21 critical region does not cause specific Down syndrome phenotypes. *Science*. 2004; 306:687–690. [PubMed: 15499018]
- Olson LE, Roper RJ, Sengstaken CL, Peterson EA, Aquino V, Galdzicki Z, Siarey R, Pletnikov M, Moran TH, Reeves RH. Trisomy for the Down syndrome ‘critical region’ is necessary but not sufficient for brain phenotypes of trisomic mice. *Hum Mol Genet*. 2007; 16:774–782. [PubMed: 17339268]
- Pereira PL, Magnol L, Sahún I, Brault V, Duchon A, Prandini P, Gruart A, Bizot JC, Chadeaux-Vekemans B, Deutsch S, Trovero F, Delgado-García JM, Antonarakis SE, Dierssen M, Hérault Y. 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*. 2009; 18:4756–4769. [PubMed: 19783846]
- Perrin S. Preclinical research: Make mouse studies work. *Nature*. 2014; 507:423–425. [PubMed: 24678540]
- Petit F, Plessis G, Decamp M, Cuisset JM, Blyth M, Pendlebury M, Andrieux J. 21q21 deletion involving NCAM2: report of 3 cases with neurodevelopmental disorders. *Eur J Med Genet*. 2015; 58:44–46. [PubMed: 25464110]

- Qi C, Chang J, Zhu Y, Yeldandi AV, Rao SM, Zhu YJ. Identification of protein arginine methyltransferase 2 as a coactivator for estrogen receptor alpha. *J Biol Chem*. 2002; 277:28624–28630. [PubMed: 12039952]
- Quiñones-Lombrana A, Blanco JG. Chromosome 21-derived hsa-miR-155-5p regulates mitochondrial biogenesis by targeting Mitochondrial Transcription Factor A (TFAM). *Biochim Biophys Acta*. 2015; 1852:1420–1427. [PubMed: 25869329]
- Rahmani Z, Blouin JL, Créau-Goldberg N, Watkins PC, Mattei JF, Poissonnier M, Prieur M, Chettouh Z, Nicole A, Aurias A, et al. Down syndrome critical region around D21S55 on proximal 21q22.3. *Am J Med Genet Suppl*. 1990; 7:98–103. [PubMed: 2149984]
- Ramos AD, Attenello FJ, Lim DA. Uncovering the roles of long noncoding RNAs in neural development and glioma progression. *Neurosci Lett*. 2015:S0304–3940. 30316–5.
- Reeves R, Irving N, Moran T, Wohn A, Kitt C, Sisodia S, Schmidt C, Bronson R, Davisson MT. A mouse model for Down Syndrome exhibits learning and behaviour deficits. *Nat genet*. 1995; 11:177–184. [PubMed: 7550346]
- Rogers MA, Langbein L, Winter H, Ehmann C, Praetzel S, Schweizer J. Characterization of a first domain of human high glycine-tyrosine and high sulfur keratin-associated protein (KAP) genes on chromosome 21q22.1. *J Biol Chem*. 2002; 277:48993–9002. [PubMed: 12359730]
- Rogers MA, Langbein L, Praetzel-Wunder S, Winter H, Schweizer J. Human hair keratin-associated proteins (KAPs). *Int Rev Cytol*. 2006; 251:209–263. [PubMed: 16939781]
- Rosenbloom KR, Sloan CA, Malladi VS, Dreszer TR, Learned K, Kirkup VM, Wong MC, Maddren M, Fang R, Heitner SG, Lee BT, Barber GP, Harte RA, Diekhans M, Long JC, Wilder SP, Zweig AS, Karolchik D, Kuhn RM, Haussler D, Kent WJ. ENCODE data in the UCSC Genome Browser: year 5 update. *Nucleic Acids Res*. 2013; 41:D56–63. [PubMed: 23193274]
- Rosenbloom KR, Armstrong J, Barber GP, Casper J, Clawson H, Diekhans M, Dreszer TR, Fujita PA, Guruvadoo L, Haeussler M, Harte RA, Heitner S, Hickey G, Hinrichs AS, Huble R, Karolchik D, Learned K, Lee BT, Li CH, Miga KH, Nguyen N, Paten B, Raney BJ, Smit AF, Speir ML, Zweig AS, Haussler D, Kuhn RM, Kent WJ. The UCSC Genome Browser database: 2015 update. *Nucleic Acids Res*. 2015; 43:D670–81. [PubMed: 25428374]
- Rueda N, Flórez J, Martínez-Cué C. Mouse models of Down syndrome as a tool to unravel the causes of mental disabilities. *Neural Plast*. 2012; 2012:584071. [PubMed: 22685678]
- Saghatelian A, Couso JP. Discovery and characterization of smORF-encoded bioactive polypeptides. *Nat Chem Biol*. 2015; 11:909–16. [PubMed: 26575237]
- Sago H, Carlson EJ, Smith DJ, Kilbridge J, Rubin EM, Mobley WC, Epstein CJ, Huang TT. Ts1Cje, a partial trisomy 16 mouse model for Down syndrome, exhibits learning and behavioral abnormalities. *Proc Natl Acad Sci U S A*. 1998; 95:6256–61. [PubMed: 9600952]
- Salehi A, Delcroix JD, Belichenko PV, Zhan K, Wu C, Valletta JS, Takimoto-Kimura R, Kleschevnikov AM, Sambamurti K, Chung PP, Xia W, Villar A, Campbell WA, Kulnane LS, Nixon RA, Lamb BT, Epstein CJ, Stokin GB, Goldstein LS, Mobley WC. Increased App expression in a mouse model of Down's syndrome disrupts NGF transport and causes cholinergic neuron degeneration. *Neuron*. 2006; 51:29–42. [PubMed: 16815330]
- Sauvageau M, Goff LA, Lodato S, Bonev B, Groff AF, Gerhardinger C, Sanchez-Gomez DB, Hacısuleyman E, Li E, Spence M, Liapis SC, Mallard W, Morse M, Swerdel MR, D'Ecclesiss MF, Moore JC, Lai V, Gong G, Yancopoulos GD, Friendewey D, Kellis M, Hart RP, Valenzuela DM, Arlotta P, Rinn JL. Multiple knockout mouse models reveal lincRNAs are required for life and brain development. *Elife*. 2013; 2:e01749. [PubMed: 24381249]
- Shi HS, Yin X, Song L, Guo QJ, Luo XH. Neuropeptide Trefoil factor 3 improves learning and retention of novel object recognition memory in mice. *Behav Brain Res*. 2012; 227:265–269. [PubMed: 22085879]
- Shin N, Lee S, Ahn N, Kim SA, Ahn SG, YongPark Z, Chang S. Sorting nexin 9 interacts with dynamin 1 and N-WASP and coordinates synaptic vesicle endocytosis. *J Biol Chem*. 2007; 282:28939–28950. [PubMed: 17681954]
- Siarey RJ, Villar AJ, Epstein CJ, Galdzicki Z. Abnormal synaptic plasticity in the Ts1Cje segmental trisomy 16 mouse model of Down syndrome. *Neuropharmacology*. 2005; 49:122–128. [PubMed: 15992587]

- Smith CM, Steitz JA. Classification of gas5 as a multi-small-nucleolar-RNA (snoRNA) host gene and a member of the 5'-terminal oligopyrimidine gene family reveals common features of snoRNA host genes. *Mol Cell Biol*. 1998; 18:6897–909. [PubMed: 9819378]
- Song J, Lee JE. miR-155 is involved in Alzheimer's disease by regulating T lymphocyte function. *Front Aging Neurosci*. 2015; 7:61. [PubMed: 25983691]
- Souchet B, Guedj F, Penke-Verdier Z, Daubigney F, Duchon A, Herault Y, Bizot JC, Janel N, Créau N, Delatour B, Delabar JM. Pharmacological correction of excitation/inhibition imbalance in Down syndrome mouse models. *Front Behav Neurosci*. 2015; 9:267. [PubMed: 26539088]
- Starbuck JM, Dutka T, Ratliff TS, Reeves RH, Richtsmeier JT. Overlapping trisomies for human chromosome 21 orthologs produce similar effects on skull and brain morphology of Dp(16)1Yey and Ts65Dn mice. *Am J Med Genet A*. 2014; 164A:1981–90. [PubMed: 24788405]
- Sturgeon X, Gardiner KJ. Transcript catalogs of human chromosome 21 and orthologous chimpanzee and mouse regions. *Mamm Genome*. 2011; 22:261–271. [PubMed: 21400203]
- Sturgeon X, Le T, Ahmed MM, Gardiner KJ. Pathways to cognitive deficits in Down syndrome. *Prog Brain Res*. 2012; 197:73–100. [PubMed: 22541289]
- Su X, Zhu G, Ding X, Lee SY, Dou Y, Zhu B, Wu W, Li H. Molecular basis underlying histone H3 lysine-arginine methylation pattern readout by Spin/Ssty repeats of Spindlin1. *Genes Dev*. 2014; 28:622–636. [PubMed: 24589551]
- Tomaselli S, Bonamassa B, Alisi A, Nobili V, Locatelli F, Gallo A. ADAR enzyme and miRNA story: a nucleotide that can make the difference. *Int J Mol Sci*. 2013; 14:22796–22816. [PubMed: 24256817]
- Vance KW, Ponting CP. Transcriptional regulatory functions of nuclear long noncoding RNAs. *Trends Genet*. 2014; 30:348–355. [PubMed: 24974018]
- Verma S, Quillinan N, Yang YF, Nakayama S, Cheng J, Kelley MH, Herson PS. TRPM2 channel activation following in vitro ischemia contributes to male hippocampal cell death. *Neurosci Lett*. 2012; 530:41–46. [PubMed: 23041043]
- Vidal V, García S, Martínez P, Corrales A, Flórez J, Rueda N, Sharma A, Martínez-Cué C. Lack of behavioral and cognitive effects of chronic ethosuximide and gabapentin treatment in the Ts65Dn mouse model of Down syndrome. *Neuroscience*. 2012; 220:158–68. [PubMed: 22728103]
- Warth SC, Hoefig KP, Hiekel A, Schallenberg S, Jovanovic K, Klein L, Kretschmer K, Ansel KM, Heissmeyer V. Induced miR-99a expression represses Mtor cooperatively with miR-150 to promote regulatory T-cell differentiation. *EMBO J*. 2015; 34:1195–213. [PubMed: 25712478]
- White R, Morganstein D, Christian M, Seth A, Herzog B, Parker MG. Role of RIP140 in metabolic tissues: connections to disease. *FEBS Lett*. 2008; 582:39–45. [PubMed: 18023280]
- Winkler GS. The mammalian anti-proliferative BTG/Tob protein family. *J Cell Physiol*. 2010; 222:66–72. [PubMed: 19746446]
- Winocur G, Roder J, Lobaugh N. Learning and memory in S100-beta transgenic mice: an analysis of impaired and preserved function. *Neurobiol Learn Mem*. 2001; 75:230–43. [PubMed: 11222062]
- Winther M, Berezin V, Walmod PS. NCAM2/OCAM/RNCAM: cell adhesion molecule with a role in neuronal compartmentalization. *Int J Biochem Cell Biol*. 2012; 44:441–446. [PubMed: 22155300]
- Worby CA, Simonson-Leff N, Clemens JC, Kruger RP, Muda M, Dixon JE. The sorting nexin, DSH3PX1, connects the axonal guidance receptor, Dscam, to the actin cytoskeleton. *J Biol Chem*. 2001; 276:41782–41789. [PubMed: 11546816]
- Wu D, Zhou Y, Pan H, Qu P, Zhou J. microRNA 99a inhibits cell proliferation, colony formation ability, migration and invasion by targeting fibroblast growth factor receptor 3 in prostate cancer. *Mol Med Rep*. 2015; 11:1469–75. [PubMed: 25352177]
- Wu N, Lin X, Zhao X, Zheng L, Xiao L, Liu J, Ge L, Cao S. MiR-125b acts as an oncogene in glioblastoma cells and inhibits cell apoptosis through p53 and p38MAPK-independent pathways. *Br J Cancer*. 2013; 109:2853–63. [PubMed: 24169356]
- Xie YF, Belrose JC, Lei G, Tymianski M, Mori Y, Macdonald JF, Jackson MF. Dependence of NMDA/GSK-3 β mediated metaplasticity on TRPM2 channels at hippocampal CA3-CA1 synapses. *Mol Brain*. 2011; 4:44. [PubMed: 22188973]

- Xu Y, Zhang HT, O'Donnell JM. Phosphodiesterases in the central nervous system: implications in mood and cognitive disorders. *Handb Exp Pharmacol*. 2011; 204:447–485.
- Yang DS, Stavrides P, Mohan PS, Kaushik S, Kumar A, Ohno M, Schmidt SD, Wesson DW, Bandyopadhyay U, Jiang Y, Pawlik M, Peterhoff CM, Yang AJ, Wilson DA, St George-Hyslop P, Westaway D, Mathews PM, Levy E, Cuervo AM, Nixon RA. Therapeutic effects of remediating autophagy failure in a mouse model of Alzheimer disease by enhancing lysosomal proteolysis. *Autophagy*. 2011; 7:788–789. [PubMed: 21464620]
- Yang QG, Wang F, Zhang Q, Xu WR, Chen YP, Chen GH. Correlation of increased hippocampal Sumo3 with spatial learning ability in old C57BL/6 mice. *Neurosci Lett*. 2012; 518:75–9. [PubMed: 22595540]
- Yang Z, Han Y, Cheng K, Zhang G, Wang X. miR-99a directly targets the mTOR signalling pathway in breast cancer side population cells. *Cell Prolif*. 2014; 47:587–95. [PubMed: 25348507]
- Yu H, et al. TCP10L is expressed specifically in spermatogenic cells and binds to death associated protein kinase-3. *Int J Androl*. 2005; 28:163–170. [PubMed: 15910542]
- Yu SH, Zhang CL, Dong FS, Zhang YM. miR-99a suppresses the metastasis of human non-small cell lung cancer cells by targeting AKT1 signaling pathway. *J Cell Biochem*. 2015; 116:268–76. [PubMed: 25187230]
- Yu T, Li Z, Jia Z, Clapcote SJ, Liu C, Li S, Asrar S, Pao A, Chen R, Fan N, Carattini-Rivera S, Bechard AR, Spring S, Henkelman RM, Stoica G, Matsui S, Nowak NJ, Roder JC, Chen C, Bradley A, Yu YE. A mouse model of Down syndrome trisomic for all human chromosome 21 syntenic regions. *Hum Mol Genet*. 2010a; 19:2780–2791. [PubMed: 20442137]
- Yu T, Liu C, Belichenko P, Clapcote SJ, Li S, Pao A, Kleschevnikov A, Bechard AR, Asrar S, Chen R, Fan N, Zhou Z, Jia Z, Chen C, Roder JC, Liu B, Baldini A, Mobley WC, Yu YE. Effects of individual segmental trisomies of human chromosome 21 syntenic regions on hippocampal long-term potentiation and cognitive behaviors in mice. *Brain Res*. 2010b; 1366:162–71. [PubMed: 20932954]
- Yu X, Li Z. Long non-coding RNA growth arrest-specific transcript 5 in tumor biology. *Oncol Lett*. 2015; 10:1953–1958. [PubMed: 26622780]
- Zenaro E, Pietronigro E, Bianca VD, Piacentino G, Marongiu L, Budui S, Turano E, Rossi B, Angiari S, Dusi S, Montresor A, Carlucci T, Nani S, Tosadori G, Calciano L, Catalucci D, Berton G, Bonetti B, Constantin G. Neutrophils promote Alzheimer's disease-like pathology and cognitive decline via LFA-1 integrin. *Nat Med*. 2015; 21:880–6. [PubMed: 26214837]
- Zhang L, Meng K, Jiang X, Liu C, Pao A, Belichenko PV, Kleschevnikov AM, Josselyn S, Liang P, Ye P, Mobley WC, Yu YE. Human chromosome 21 orthologous region on mouse chromosome 17 is a major determinant of Down syndrome-related developmental cognitive deficits. *Hum Mol Genet*. 2014; 23:578–589. [PubMed: 24041763]
- Zhao B, Han H, Chen J, Zhang Z, Li S, Fang F, Zheng Q, Ma Y, Zhang J, Wu N, Yang Y. MicroRNA let-7c inhibits migration and invasion of human non-small cell lung cancer by targeting ITGB3 and MAP4K3. *Cancer Lett*. 2014; 342:43–51. [PubMed: 23981581]
- Zheng Z, Cai C, Omwancha J, Chen S-Y, Baslan T, Shemshedini L. SUMO-3 enhances androgen receptor transcriptional activity through a sumoylation-independent mechanism in prostate cancer cells. *J Biol Chem*. 2006; 281:4002–12. [PubMed: 16361251]
- Zhou B, Cai Q, Xie Y, Sheng ZH. Snapin recruits dynein to BDNF-TrkB signaling endosomes for retrograde axonal transport and is essential for dendrite growth of cortical neurons. *Cell Rep*. 2012; 2:42–51. [PubMed: 22840395]
- Zhou Y, Zhang X, Klibanski A. MEG3 noncoding RNA: a tumor suppressor. *J Mol Endocrinol*. 2012; 48:R45–53. [PubMed: 22393162]

Hsa21q

| | Total Hsa21q | TF | Ubi | RNA | AD | KRTAP | Conserved in Mmu | |
|--|-----------------|------|-----|-----|-----|-------|---------------------|----------|
| | 105 | 13 | 6 | 2 | 3 | 33 | 102 | Mmu16 |
| | (93) | (12) | (4) | (1) | (3) | (33) | (90) | (Ts65Dn) |
| | 21 | 2 | 1 | 2 | 0 | 0 | 19 | Mmu17 |
| | 37 | 1 | 1 | 3 | 5 | 16 | 37 | Mmu10 |

Figure 1. Distribution of protein coding genes within 21q

A schematic of a Giemsa banded Hsa21q is shown at the left. The total number of (non-Krtap) protein coding genes and numbers within several functional classes are provided for syntenic regions on mouse chromosome 16 (Mmu16), Mmu17 and Mmu10. Syntenic regions are demarcated by horizontal solid or dashed lines; similar numbers for the sub region of Mmu16 trisomic in the Ts65Dn are shown shaded. TF, transcription factors; Ubi, members of the ubiquitin pathway; RNA, proteins involved in RNA processing; AD, proteins directly relevant to Alzheimer's Disease or involved in processing or trafficking of the Hsa21 encoded protein APP (amyloid precursor protein). For functional annotations, see Supplementary Table S1.

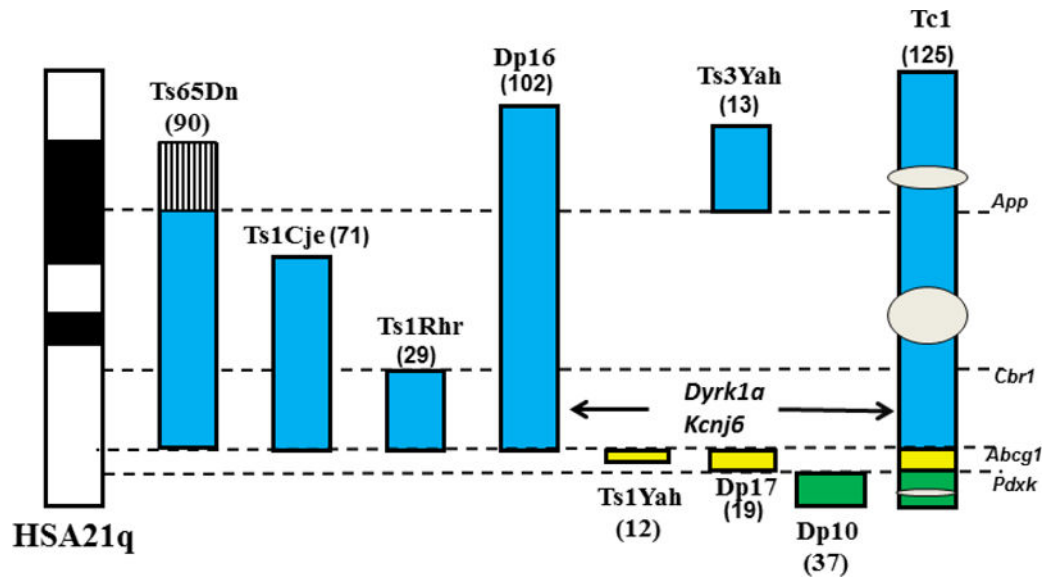


Figure 2. Trisomic regions and number of non-KRTAP protein coding genes trisomic in mouse models of DS

Blue, yellow, green: Hsa21 syntenic regions on Mmu16, 17 and 10, respectively. Numbers in brackets indicate the number of conserved protein coding genes that are trisomic. The Hsa6 syntenic region in the Ts65Dn is indicated by stripes and contains 35 protein coding genes. Grey ovals within the Tc1 chromosome indicate major deletions. Arrows indicate location of *Dyrk1A* and *Kcnj6* within Mmu16.

Table 1
Distribution within Hsa21 of protein coding and non-protein coding genes

Data were retrieved from VEGA (http://vega.sanger.ac.uk/Homo_sapiens/Location/Chromosome?r=21) (December 14, 2015). KRTAP genes were identified from gene descriptions.

| Hsa21 genes | 21q | 21p | Total |
|--------------------------------------|------------|-----------|------------|
| "Known" protein coding | 164 | 3(+11)* | 167 |
| Novel protein coding | 5 | 1 | 6 |
| Keratin-associated proteins (KRTAPs) | 49 | 0 | 49 |
| Total protein coding | 218 | 4 | 222 |
| Non-protein coding | 280 | 45 | 325 |
| Total | 498 | 49 | 547 |

* 11 protein coding genes mapped to Hsa21p in Vega were identified by BLASTN to be copies/duplications of known protein coding genes within Hsa21q22.3

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Table 2

Gene content of DS mouse models

Total Mb: genomic size of the region of Hsa21 trisomic in each model. Start-stop, Mb and protein coding: the orthologous region of Hsa21 spanned in each model. Protein coding genes (non-KRTAPs): total number in the trisomic region, and the number conserved between human and mouse. RNA genes: number of lncRNA present in the human and mouse regions spanned in each model, and conserved between human and mouse (by nucleotide sequence similarity). Numbers were obtained for human from VEGA (December 14, 2015) and for mouse from Gencode/Encode (Human Genome Browser) and Sturgeon and Gardiner 2011.

| | Total Mb | Start-stop, Mb | Start-stop, protein coding | Protein coding | | RNA genes | | |
|-------------|----------|----------------|----------------------------|----------------|-----------|-----------|-------|-----------|
| | | | | Total | conserved | Human | Mouse | conserved |
| 21p | 10.6 | 1–10.6 | BAGE2-TPTE | 2 | 0 | 45 | 0 | |
| 21q | 33.7 | 13.0–46.7 | POTED-PRTM2 | 164 | 158 | 280 | 115 | 75 |
| Tc1 | 42 | na | LIPI-PRMT2 | 129 | 125 | 243 | 91 | 61 |
| Prox Mmu16 | 1.1 | 13.0–14.1 | POTED | 1 | 0 | 5 | 0 | 0 |
| Dp16 | 27.9 | 14.1–42.0 | LIPI-ZBTB21 | 105 | 102 | 200 | 91 | 61 |
| Ts3Yah | 11.8 | 14.3–26.1 | SAMSN1-GABPA | 13 | 13 | 75 | 38 | 24 |
| Prox Ts65Dn | 11.4 | 14.1–25.5 | LIPI-> MIR155HG | 12 | 12 | 74 | 36 | 24 |
| Ts65Dn | 16.4 | 25.6–42.0 | MRPL39-ZBTB21 | 93 | 90 | 125 | 55 | 37 |
| Ts1Cje | 10.3 | 31.7–42.0 | >SOD1-ZBTB21 | 74 | 71 | 92 | 43 | 28 |
| Ts1Rhr | 5.3 | 36.1–41.4 | CBR1-FAM3B | 31 | 29 | 44 | 14 | 10 |
| Ts1Yah | 0.7 | 42.3–43.0 | TFE3-CBS | 12 | 12 | 11 | 4 | 0 |
| Dp17 | 1.7 | 42.1–43.8 | UMODL1-RRP1B | 21 | 19 | 19 | 6 | 0 |
| Dp10 | 2.9 | 43.8–46.7 | PDXK-PRMT2 | 37 | 37 | 56 | 18 | 14 |

Table 3
Functional and phenotypic interactions among syntenic mouse regions

MWM, Morris water maze; YM, Y maze; TM, T-maze; NOR, novel object recognition; CFC, context fear conditioning; LTP, long term potentiation. Normal, not different from wild type littermate controls; improved, less impaired than Dp16 but not to level of wild type controls; enhanced, increased relative to controls

| | MWM | YM | TM | NOR | CFC | LTP | References |
|----|----------|----------|----------|----------|----------|----------|---|
| 1 | impaired | impaired | | | | elevated | Ahn et al 2006; Souchet et al 2015 |
| 2 | normal | | impaired | impaired | | | Olsen et al 2007; Belichenko et al., 2009 |
| 3 | impaired | | impaired | normal | | impaired | Starey et al 2005; Olsen et al 2007 |
| 4 | impaired | | impaired | | impaired | impaired | Yu et al 2010b; Zhang et al 2014 |
| 5 | | | improved | | improved | improved | Jiang et al 2015 |
| 6 | | | | | impaired | normal | Cooper et al 2012 |
| 7 | | | impaired | | impaired | impaired | Jiang et al 2015 |
| 8 | normal | | improved | | improved | improved | Zhang et al 2014; Jiang et al 2015 |
| 9 | impaired | | | | | impaired | Zhang et al 2014 |
| 10 | enhanced | impaired | | impaired | | enhanced | Pereira et al 2009 |
| 11 | normal | | | | normal | enhanced | Yu et al 2010b |
| 12 | normal | | | | normal | normal | Yu et al 2010b |
| 13 | normal | | | | | normal | Zhang et al 2014 |
| 14 | impaired | impaired | impaired | impaired | impaired | impaired | Rueda et al 2012 |
| 15 | normal | | | | | normal | Olsen et al 2007 |
| 16 | improved | | | | improved | normal | Garcia-Cerro et al 2014 |

Table 4

Functions of selected Hsa21 proteins with orthologs trisomic in the Dp10.

| Protein | Functional description | Reference |
|---------|---|---|
| PRMT2 | Protein Arg methyl-transferase. Substrates & consequences: activation of estrogen, progesterone and androgen receptors; histone H3 and regulation of Wnt signaling, β -catenin signaling; STAT3 phosphorylation | Qi et al. 2002; Meyer et al. 2007; Blythe et al 2010; Iwasaki et al. 2010; Su et al. 2014 |
| SUMO3 | Small ubiquitin-like modifier; increased levels in aged mice correlate with LM. Overexpression affects A β production. Indirect modulation of estrogen, progesterone and androgen receptors | Li et al. 2003; Zheng et al. 2006; Yang et al. 2012 |
| TRPM2 | Ca-permeable cation channel; activation by oxidative stress confers susceptibility to cell death. Inhibition protects from ischemia through modulation of NMDA receptor subunit expression. Knockout protects males but not females from effects of ischemia. Activation by A β contributes to vascular pathology in AD. Activity modulated by melatonin. | Jia et al. 2011; Xie et al. 2011; Celik and Naziro lu 2012; Verma et al. 2012; Alim et al. 2013 |
| ADARB1 | Adenosine deaminase (guanosine to inosine editing) of mRNAs and miRNAs. Substrates include Gli1 (hedgehog signaling), and serotonin 2C, GABAA3 and glutamate receptors. Knockout affects acoustic startle response in males but not females. | Horsch et al. 2011; Tomaselli et al. 2013 |
| FTCD | Formimidoyltransferase cyclodeaminase. Mutations have been implicated in glutamate formiminotransferase deficiency, characterized by intellectual disability | Hilton et al. 2003 |
| S100B | Ca-binding protein. Neuroprotective or neurotoxic depending on concentration and localization. Synergistic function with APP to impair neurogenesis; exacerbates phenotype in AD mouse model; overexpression causes L/M deficits and neurodegeneration that are exacerbated by anti-oxidants | Winocur et al. 2001; Bialowas-McGoey et al. 2008; Donato et al. 2009; Mori et al. 2010; Lu et al. 2011; |
| COL18A1 | Collagen 18A1 proteolytically cleaved to endostatin. Endostatin inhibits neurite outgrowth; required for cerebellar development. Accumulates in AD plaques | Deiningner et al. 2002; Al Ahmad et al. 2010 |
| CSTB | Lysosomal cysteine-protease inhibitor; mutated in epilepsy; CSTB inhibition in AD mouse model improves A β clearance and prevents L/M deficits | Yang et al. 2011 |
| ITGB2 | B2-integrin. Reduces A β accumulation; contributes to neuroinflammation in Tauopathy. | Choucair-Jaafar et al. 2011; Zenaro et al. 2015 |