Title

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Journal

British Medical Bulletin, 120(1)

ISSN

0007-1420

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Publication Date

2016-12-01

DOI

10.1093/bmb/ldw040

Peer reviewed

British Medical Bulletin, 2016, **120**:111–122
doi: 10.1093/bmb/ldw040
Advance Access Publication Date: 25 October 2016

Invited Review

Mouse-based genetic modeling and analysis of Down syndrome

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Editorial Decision 8 September 2016; Accepted 3 October 2016

Abstract

Introduction: Down syndrome (DS), caused by human trisomy 21 (Ts21), can be considered as a prototypical model for understanding the effects of chromosomal aneuploidies in other diseases. Human chromosome 21 (Hsa21) is syntenically conserved with three regions in the mouse genome.

Sources of data: A review of recent advances in genetic modeling and analysis of DS. Using Cre/*loxP*-mediated chromosome engineering, a substantial number of new mouse models of DS have recently been generated, which facilitates better understanding of disease mechanisms in DS.

Areas of agreement: Based on evolutionary conservation, Ts21 can be modeled by engineered triplication of Hsa21 syntenic regions in mice. The validity of the models is supported by the exhibition of DS-related phenotypes.

Areas of controversy: Although substantial progress has been made, it remains a challenge to unravel the relative importance of specific candidate

genes and molecular mechanisms underlying the various clinical phenotypes.

Growing points: Further understanding of mechanisms based on data from mouse models, in parallel with human studies, may lead to novel therapies for clinical manifestations of Ts21 and insights to the roles of aneuploidies in other developmental disorders and cancers.

Key words: Down syndrome, human trisomy 21, mouse models, chromosome engineering

Introduction

Human trisomy 21 (Ts21, Down syndrome, DS) is the most common chromosomal abnormality compatible with postnatal survival and occurs in one in ~691 and 1000 newborns in the USA[1] and Europe,[2] respectively. It is a leading genetic cause of congenital heart disease, acute megakaryoblastic leukemia and developmental cognitive deficits. It causes early onset Alzheimer-type neurodegeneration in nearly every individual with DS. The pregnancy termination rate after prenatal diagnosis of human Ts21 has not increased and the incidence rate of DS has not decreased in the last decade in countries like the USA.[3] Among a constellation of DS phenotypes, some of them, such as developmental cognitive deficits and Alzheimer's disease (AD), impact both the affected individuals and their families, and are without effective treatments. After the discovery that individuals with DS carry an extra copy of human chromosome 21 (Hsa21),[4,5] a subsequent major effort was to try to define subgenomic regions associated with various DS phenotypes by examining human segmental trisomies. In these experiments, data generated from individuals with segmental trisomy of Hsa21 were used to establish genotype–phenotype relationships.[6–8] However, interpretation of these studies is not straightforward because some individuals carrying segmental Ts21 also have additional genomic abnormalities, such as unbalanced derivatives associated with non-Hsa21 genomic regions stemming from chromosomal translocations.[6–8] Another inherent problem is that the endpoints of segmental trisomies are almost always unique among the cases. Therefore, for almost any segmental trisomy case, the sample size for a specific genotype is one, which is a major obstacle in distinguishing the contributions of trisomy, versus unique characteristics of a given subject, to a phenotype.[9] For these reasons, research efforts have turned to well-controlled model organisms, particularly the mouse, to unravel the biology associated with DS.

Modeling DS at the early stage

Based on the findings that many Hsa21 gene orthologs mapped to mouse chromosome 16 (Mmu16), the first trisomic model of DS was mouse trisomy 16.[10,11] However, this mutant, with the entire extra chromosome 16, is embryonic lethal and thus many important postnatal phenotypes of DS cannot be studied. Therefore, the discovery of postnatally viable Ts65Dn mice was considered as a major development in DS research.[12] Ts65Dn mice carry an unbalanced derivative, Ts(17^{16})65Dn, of a balanced translocation, which was randomly induced by irradiation.[12] The Ts(17^{16})65Dn chromosome consists of the entire genomic region distal to *Mir155* on Mmu16 and a subcentromeric region on Mmu17, which is not syntenic to Hsa21[13,14] (Supplementary Table S1). The second postnatally viable mouse model of DS is Ts1Cje, which carries an unbalanced derivative, Ts(12^{16})1Cje, of a balanced translocation, which was induced by gene-targeting in mouse ES cells.[15] The Ts(12^{16})1Cje chromosome carries the entire genomic region distal to *Sod1* on Mmu16 with *Sod1* inactivated[13,15] (Supplementary Table S1). Recent analyses showed a heterozygous deletion on Mmu12 in Ts1Cje mice, which is not syntenic to

Hsa21.[13,16] The phenotypes of Ts65Dn and Ts1Cje mice have been extensively characterized and, although these mice are not perfect molecular mimics of human DS, they do show many phenotypic features of the human syndrome[17–21] (Supplementary Table S2).

Transchromosomal mouse models of DS

Another strategy to model DS is to generate mice carrying an actual Hsa21—thus, a 'transchromosomal model'. Using microcell-mediated chromosome transfer, Hsa21 segments and an entire Hsa21 were introduced to mouse ES cells and mouse mutants were then generated using these cells.[22–25] Among transchromosomal models, Tc1 mice carry more Hsa21 genetic materials than any other transchromosomal mouse models of DS. Probably because Hsa21 was irradiated before being transferred to mouse ES cells, the Hsa21 in Tc1 mice carries genetic alterations, including deletions, duplications and other rearrangements.[26] The Tc1 mice have been extensively characterized and, like the models in the previous section, despite the presence of secondary molecular aberrations, they too show several phenotypic features similar to human DS[25,27] (Supplementary Table S2).

Genetic modeling and dissection of DS using mouse mutants generated by Cre/*loxP*-mediated chromosome engineering

Ts65Dn and Ts1Cje mice are important viable trisomic mouse models. However, neither is a complete model. The comparison between the human and mouse genomes revealed that the regions of Hsa21 are syntenically conserved in three regions in the mouse genome located on Mmu10, Mmu16 and Mmu17 (Fig. 1) (www.ensembl.org). Only <65% of Hsa21 gene orthologs are triplicated in Ts65Dn and Ts1Cje mice. Because these models were discovered by serendipity, it will be difficult to generate additional models of DS trisomic for different Hsa21 syntenic regions by the procedures

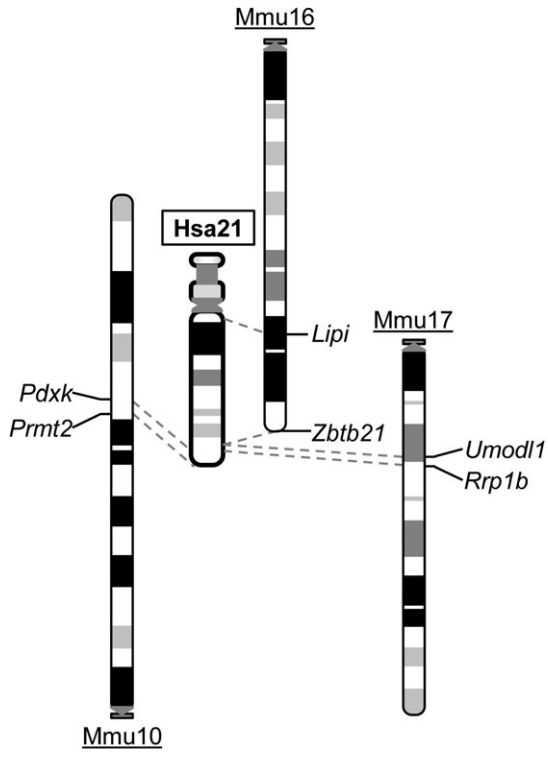


Fig. 1 Shared syntenies between Hsa21 and three regions in the mouse genome which are located on Mmu10, Mmu16 and Mmu17. The endpoints of the syntenic regions in mice are indicated.

used for generating Ts65Dn and Ts1Cje mice. This difficult technical obstacle was finally overcome by the development of Cre/*loxP*-mediated chromosome engineering technology. This technology can be used to generate chromosomal duplications and deletions with predetermined endpoints via three steps.[28] First, a *loxP* site is introduced into the first endpoint in the ES cell genome with a positive selection marker (Fig. 2). One of such positive selection markers is the neomycin resistance gene (*neo*). When G418, an antibiotic, is added to the culture medium, the cells expressing *neo* survive and are selected for. Next, a second *loxP* site is targeted to a second endpoint with an alternative positive selection marker such as the puromycin resistance gene. To induce recombination, a Cre expression vector is electroporated into double-targeted clones. If two targeted *loxP*s are located on the same chromosome homolog (*in cis*) and oriented in the same direction

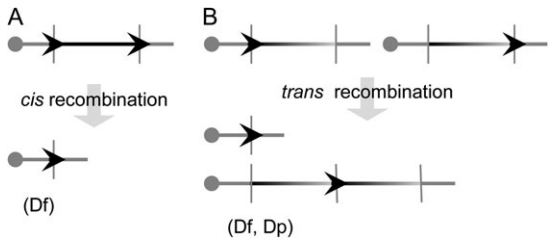
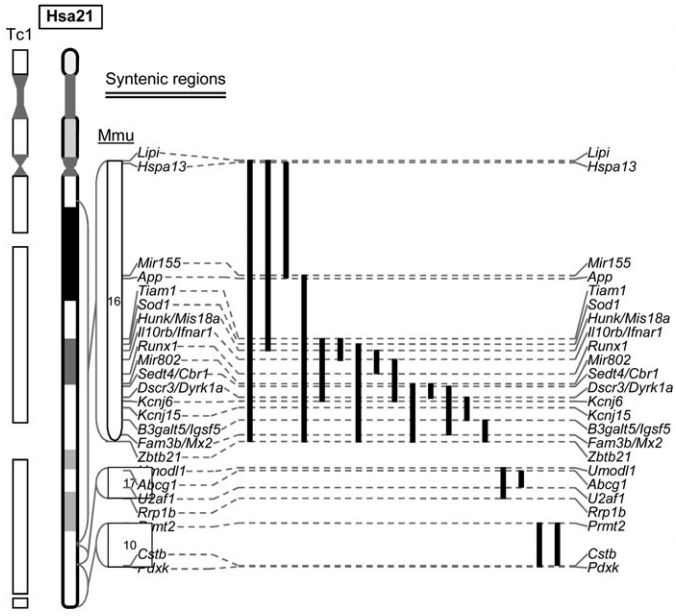


Fig. 2 The strategy to generate deletions and duplications in mouse ES cells using Cre/*loxP*-mediated chromosome engineering.[28] To generate Dp and Df, *loxP* is inserted into two endpoints of an orthologous region of Hsa21 in the genome of mouse ES cells with two different positive selection markers, such as the neomycin and puromycin resistance genes. A Cre expression vector is then electroporated into the double-targeted cells to induce recombination. (A) If two *loxP* sites are located *in cis* and orientated in the same direction in relationship to the centromere, the recombination will result in a Df. (B) If two *loxP* sites are located *in trans* and orientated in the same direction in relationship to the centromere, the recombination will result in a Dp and a Df. The genotypes of engineered ES cells are confirmed by Southern blot analysis and fluorescence *in situ* hybridization. Afterwards, these cells are used to generate chimeras by injecting them into blastocysts. Germline transmission will lead to establishment of mouse mutants carrying a desired Dp or Df. Arrow head, *loxP*.

with respect to the centromere, recombination will result in a chromosomal deletion (deficiency, Df) (Fig. 2A). If two *loxP*s are located on two homologs (*in trans*) and oriented in the same direction, the recombination will result in a deletion (i.e. Df) and the reciprocal duplication (i.e. Dp) (Fig. 2B). The correct orientation of *loxP* on a chromosome can be achieved by choosing the desired orientation of the *loxP* in a targeting vector based on the genomic sequence. Clones carrying a desired rearrangement can be identified by analyzing the sib-selection result through positive selection drugs, and by analyzing recombination efficiencies,[28] and the rearrangements can be verified by Southern analysis and fluorescence *in situ* hybridization. Chimeras are generated by injecting the ES cells confirmed to have the rearrangements of interest into mouse blastocysts, from which the progeny that carry the rearrangements are derived. Precise rearrangements of Hsa21 syntenic regions in mouse mutants can be verified by array-based comparative genome hybridization, and most recently by inferring DNA copy number data from NextGen sequencing.

The rate-limiting factor in the aforementioned procedure is the time needed for cloning to construct targeting vectors. To improve efficiency, genomic libraries with pre-made targeting vectors were developed.[29] The vectors from these libraries contain all the required genetic elements. Approximately 153 000 λ phage clones from these libraries were converted into the plasmid form. The end sequences of their genomic inserts were derived using sequencing primers located external to the cloning sites. Based on these sequences, the genome coordinates of the genomic inserts were determined. It is estimated that for every ~39 kb of the genome at least one targeting vector is available for endpoint targeting.[30] The libraries have been designated the Mutagenic Insertion and Chromosome Engineering Resource (MICER). MICER substantially eliminates the need for constructing new targeting vectors for generating mutant mice carrying large genomic rearrangements. These vectors, which are available from 'Source BioScience', thus markedly accelerate progress in generating the desired mice. Besides engineering desired chromosomal rearrangements in ES cells and using these cells to generate mouse mutants, alternative strategies include first generating mouse mutants carrying a single targeted *loxP* at either desired endpoint. Afterwards, appropriate crossings of the mice carrying two targeted *loxP* sites with a transgenic *cre* mice, such as *Sycp1-cre* or *Tg(Pgk1-cre)1Lni* mice,[31–33] will result in Cre/*loxP*-mediated trans-recombination in the compound mutants and lead to Dp and Df in the progeny.

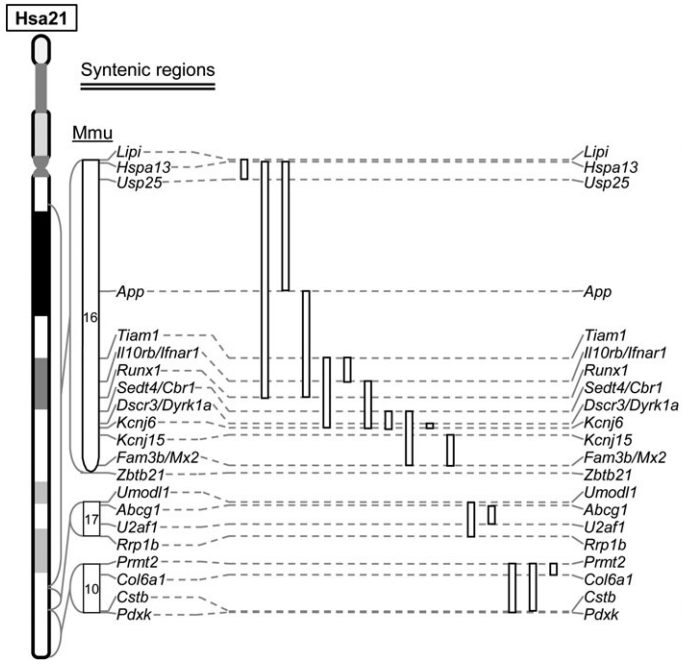
Using these procedures, several laboratories have generated a substantial number of chromosomal rearrangements in Hsa21 syntenic regions in mice (Figs 3 and 4). The duplication mutants have been used to determine if the triplication of the entire Hsa21 syntenic regions on Mmu10, Mmu16 and/or Mmu17 as well as a sub-segment within a Hsa21 syntenic region is sufficient to cause a DS-related phenotype.[34–38] To determine if a given relatively small region is necessary for a phenotype, a subtractive strategy can be used by compounding a larger duplication with a deletion of the smaller sub-region.[33,39–42] Using different combinations of



Mouse models with 3 copies of Hsa21 orthologous genes

Mutant alleles	Endpoints	# of orthologous genes
Tc1[25,26]		200
Dp(16)1Yey[34]	*Lipi-Zbtb21*	118
Dp1TybEmcf[75]	*Lipi-Zbtb21*	118
Dp9TybEmcf[75]	*Lipi-Hunk*	50
Ts2Yah[37,38,105]	*Hspa13-App*	19
Ts65Dn[12]	*Mir155-Zbtb21*	100
Dp(16)2Yey[73]	*Tiam1-Kcnj6*	51
Dp(16)3Yey[74]	*Tiam1-Il10rb*	16
Ts1Cje[15]	*Sod1-Zbtb21*	70
Dp2TybEmcf[75]	*Mis18a-Runx1*	31
Dp(16)4Yey[74]	*Ifnar1-Kcnj6*	35
Dp3TybEmcf[75]	*Mir802-Zbtb21*	37
Dp4TybEmcf[75]	*Mir802-Dscr3*	14
Ts1Rhr[106]	*Cbr1-Fam3b*	29
Dp5TybEmcf[75]	*Dyrk1a-B3galt5*	12
Dp6TybEmcf[75]	*Igsf5-Zbtb21*	11
Dp(17)1Yey[68,69]	*Umodl1-Rrp1b*	19
Ts1Yah[36,105]	*Abcg1-U2af1*	14
Dp(10)1Yey[68,69]	*Prmt2-Pdxk*	39
Ts3Yah[105]	*Prmt2-Cstb*	38

Fig. 3 Mouse mutants which carry a triplication of a Hsa21 syntenic region. A solid line represents the region triplicated for a Hsa21 syntenic region in a mouse model listed in the table.



Mouse mutants deficient for a Hsa21 syntenic region

Mutant alleles	Endpoints	# of orthologous genes
Ms1Dja[107]	*Lipi-Usp25*	6
Ms6Yah[105]	*Hspa13-Runx1*	79
Ms3Yah[38,105]	*Hspa13-App*	20
Ms5Yah[33,105]	*App-Runx1*	61
Df(16)2Yey[73]	*Tiam1-Kcnj6*	51
Df(16)3Yey[74]	*Tiam1-Il10rb*	16
Df(16)4Yey[74]	*Ifnar1-Kcnj6*	35
Df(16)5Yey[41]	*Setd4-Kcnj6*	15
Ms1Rhr[106]	*Cbr1-Fam3b*	29
Df(16)7Yey[41]	*Dyrk1a-Kcnj6*	2
Df(16)6Yey[41]	*Kcnj15-Mx2*	16
Df(17)1Yey[108]	*Umodl1-Rrp1b*	19
Ms2Yah[36,105]	*Abcg1-U2af1*	14
Df(10)1Yey[108]	*Prmt2-Pdxk*	39
Ms4Yah[40,105]	*Prmt2-Cstb*	38
Ms1Yah[105,109]	*Prmt2-Col6a1*	12

Fig. 4 Mouse mutants which carry a deletion of a Hsa21 syntenic region. An open line represents the region deleted for a Hsa21 syntenic region in a mouse model listed in the table.

Dp and Df mutants, the smallest genomic region can be identified for a specific DS phenotype. If this region contains 10 or more Hsa21 gene orthologs, it might be necessary to generate new Dp and/or Df mutants to further dissect the region. After a minimal critical genomic region is identified, a compound mutant could be generated to carry a duplication of the region and a null allele of the gene located within the region. The contribution of the gene to the phenotype could be established based on elimination or significant alleviation of a DS-related phenotype observed in a mouse mutant carrying the duplication alone. This type of 'subtractive' or 'normalization' strategy has been used by many labs to ascertain the contribution of individual Hsa21 genes orthologs.[43–47] For this reason, mouse mutants carrying null alleles of Hsa21 gene orthologs are essential reagents for examining the contributions of these genes to DS phenotypes. Through the years, null alleles for many of these genes were generated by individual laboratories because of their importance as individual genes. The pace of null allele generation was drastically expedited after the launches of several systematic knockout (KO) projects, including 'NIH Knockout Mouse Project' and 'The European Conditional Mouse Mutagenesis Program'.[48–51] The current status of the KO mice for the Hsa21 gene orthologs in the public domain, including the status of targeting vector, targeted ES cells and mouse mutants, is presented in Supplementary Table S1.

Impact of Ts21 on DNA methylation patterns

Evidence has been continuously accumulating that the dosage increase of a Hsa21 gene or gene ortholog can contribute to a mutant phenotype associated with DS. Recent results suggest the possibility that epigenetic events may also be involved in such genotype–phenotype relationships. Gene-specific alterations in CpG methylation were first detected in blood leukocytes from adults with DS when compared to the samples from control individuals.[52] The presence of such a DS-specific methylation profile was further supported by other studies on the samples isolated from Ts21 placentas, fibroblasts[53–55] and more recently neural tissues.[56] Interestingly, such a phenomenon was recapitulated in mouse models of DS,[56] providing a system for further exploring the processes and the consequences of DS-associated methylation alterations. Moreover, the epigenetic responses to the presence of the extra genetic material are not restricted to altered CpG methylation patterns; changes in histone modifications have also been described.[57] When considered as a well-defined and experimentally accessible model system, results from these studies of genetic–epigenetic interactions in human Ts21 and in the mouse lines with engineered genomic rearrangements will likely have important implications for understanding analogous genetic–epigenetic interactions in other developmental disorders associated with aneuploidies as well as in human cancers in which aneuploidies are often a hallmark.[58]

Phenotypic analysis of mouse models of DS

Developmental cognitive deficits are the most studied phenotype of DS because human Ts21 is a leading genetic cause of this phenotype.[18,59–63] The average IQ of individuals with Ts21 is significantly lower when compared with individuals without Ts21,[60,64] and while there are marked inter-individual variations, probably due to genetic background effects, some degree of intellectual disability is seen in all individuals with DS. Cognitive deficits include impairment in spatial memory and long-term memory as well as difficulties in acquiring new skills.[61,65,66] Neuropsychological examinations have revealed that individuals with Ts21 exhibit hippocampal dysfunctions.[61,67]

Since developmental cognitive deficits of Ts65Dn, Ts1Cje and Tc1 mice have been extensively reviewed, here we will focus on this phenotype analyzed in newly engineered triplication mouse models. To determine if the triplication of a specific Hsa21 syntenic region, including an entire Hsa21 sytenic region on a mouse chromosome, is necessary and/or sufficient to cause developmental cognitive deficits, cognitively relevant phenotypes of

duplication mutants and/or compound mutants were characterized, which include T-maze test, Morris water maze tests and fear conditioning tests as well as analysis of synaptic plasticity using extracellular recording of hippocampal slices. In parallel with Ts65Dn mice, abnormal cognitively relevant phenotypes were observed in *Dp(16)1Yey*/+ mice[68] and any compound mutants carrying *Dp(16)1Yey* and a duplication(s) of any other Hsa21 syntenic regions,[69,70] which include *Dp(10)1Yey*/+;*Dp(16)1Yey*/+;*Dp(17)1Yey*/+ mice carrying duplications of the entireties of all the three syntenic regions located on Mmu10, Mmu16 and Mmu17. Although Hsa21 syntenic region on Mmu10 contains 39 Hsa21 gene orthologs, a duplication of this syntenic region alone has not been shown to cause an abnormal cognitively relevant phenotype.[68] Interestingly in view of the small chromosomal sub-region involved, a duplication of the Hsa21 syntenic region on Mmu17 was able to consistently cause abnormal hippocampal long-term potentiation.[36,68] Many deletion mutants have been used to determine if a Hsa21 syntenic region is necessary for a phenotype. Such an approach has been used to show that the so-called DS critical region is necessary for cognitive deficits in young adult mice.[39,41] The extension of such a subtractive strategy has also led to show that the triplications of some Hsa21 gene orthologs are necessary for cognitively relevant phenotypes, including *Dyrk1a*.[41,71] The data from this type of analysis also suggest potential interactions between different triplicated Hsa21 gene orthologs.[36,72]

Besides developmental cognitive deficits, other phenotypes of DS that have also been analyzed in new models include heart defects,[33,73–75] craniofacial abnormalities,[76] leukemia[77] and middle ear infection.[37]

Another key phenotype in DS is AD, which is early onset with AD-type neurodegeneration detected by age 40 for all the individuals carrying Ts21.[78] The neuropathological findings of AD in DS are very similar to AD without Ts21 in the pattern of emergence of specific pathological markers, which include neuritic plaques and neurofibrillary tangles.[79–81] Neuron loss is present in the locus coeruleus and basal forebrain.[82–84] The evidence from individuals carrying segmental Ts21 suggests the triplication of the amyloid beta precursor protein gene (*APP*) is necessary for Alzheimer-type neurodegeneration.[85–89] This is also consistent with a more recent report, in which a study of 30 people partially trisomic for Hsa21 provided evidence that an increased dose of *APP* is necessary for AD in DS.[8] Interestingly, mosaic Ts21 or segmental Ts21 has recently been detected in sporadic AD cases.[90–94] Detections of mosaic wild-type *APP* triplication in brains of patients with sporadic AD suggest the possible causative relationship between APP dosage increase and neurodegeneration.[95] Therefore, AD pathogenesis in DS may provide insights into AD pathogenesis in other populations, including sporadic AD.[96,97]

Mouse models of DS have demonstrated important parallels with AD in DS. In Ts65Dn mice, age-related neurodegeneration impacts neurons of the locus coeruleus and cholinergic neurons in the basal forebrain medial septum.[44,98] Significantly, increased *App* dose was shown to be necessary for degeneration of both neuronal populations in Ts65Dn mice.[44,98] Interestingly, both the temporal and spatial patterns of neurodegeneration are also consistent with those in AD with or without Ts21; degeneration of locus coeruleus neurons predates basal forebrain cholinergic neurons.[99] Specifically, in Ts65Dn mice, locus coeruleus showed progressive age-related changes in volume and cell number at 3–6 months of age, with changes in basal forebrain cholinergic neurons at 9–12 months.[44,100–103] Another important AD neuropathology is enlargement of early endosomes. The implication is associated with the fact that APP processing occurs in endosomes.[104] One of the consequences of the abnormalities associated with endosomes is impaired retrograde trafficking of neurotrophins in endosomes of axons, which has been implicated as the cause for degeneration of basal forebrain cholinergic neurons.[44] Evidence has shown that the triplication of the *App* ortholog is required for mutant mice to exhibit enlargement of endosomes and impaired neurotrophin transport.[43,44] Together, these findings suggest that neuronal degeneration relevant to AD in DS is recapitulated in mutant mice.

Future prospects

Fueled by sequencing the human and mouse genomes and development of chromosome engineering technology, the mouse has continued to serve as a rewarding organism for genetic modeling and dissection of DS. With the development of new genome manipulating tools, such as CRISPR/Cas9, the pace of mouse-based genetic studies of DS is anticipated to be further accelerated. With null alleles of Hsa21 gene orthologs in combination with new mouse mutants carrying a duplication or deletion of Hsa21 syntenic regions, we expect to define the contributions of Hsa21 genes to various DS phenotypes, including at the behavioral, physiological, cellular and epigenetic levels, which will lay the groundwork to unravel the true mechanisms underlying these phenotypes. All these efforts are a prelude to building a sufficient knowledge basis for rational designing of therapeutic interventions to enhance the quality of life for individuals with DS.

Supplementary material

Supplementary material is available at *BRIMED* online.

Funding

The work at the authors' laboratories is supported in part by grants from the National Institutes of Health (R01NS66072, R01HL91519, P01HD35897, R21GM114645 and P30CA16056) and the Children's Guild Foundation.

Conflict of interest

The authors have no potential conflicts of interest.

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