

## Review Article

### Molecular Aspects of Down Syndrome

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*Molecular aspects of Down syndrome (DS), a major genetic cause for mental retardation, commonly associated with trisomy 21 are discussed. Two different hypotheses have been speculated to better understand the disease. One believes that increased gene dosage contributes to the phenotypic abnormalities; the other correlates genetic imbalance with DS pathogenesis. To sustain these hypotheses, different murine models have been developed. Experimental models as well as sequencing of human chromosome 21 helped in speculating a few possible candidate genes for DS. However, the phenotypic changes involved with this neurological disorder vis-a-vis the enhanced number of genes, still remain unexplained. Improvement in screening pattern, model system, as well as better understanding of the disease etiology may help in developing efficacious therapeutic regimes for DS.*

**Key words:** Candidate gene, Down syndrome, MR.

Down Syndrome (DS) is the most common genetic disorder(1) affecting 1/800 live births irrespective of gender, ethnic or

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racial group(2). Trisomy 21, partial trisomy or a Robertsonian translocation is implicated to cause DS(3). Overall estimation of trisomy 21 in DS population, possibly caused by meiotic non-disjunction, is about 95%(3). In 2 to 4% of cases, there is recognizable mosaicism of trisomic and normal cell lines(3), presumably resulting from a mitotic error in the growing embryo. In about 2% of DS patients, 1 copy of chromosome 21 is translocated to another acrocentric chromosome, most often chromosome 14 or 21(3).

#### Molecular aspects

Trisomy 21 has been hypothesized to be responsible for most of the physical features of DS. A transcription map around the marker D21S55 has been constructed(4); however, region outside the marker loci have also been found to be associated with some atypical features of DS.

Two parallel hypotheses have been proposed to explain the etiology of DS. According to the developmental instability hypothesis(5), loss of chromosomal balance causes DS; on the other hand, gene dosage hypothesis tends to correlate gene over-expression with DS etiology(5).

The chromosome 21 contains 225 genes(6), some of which, located at the Down Syndrome Critical region (DSCR), are thought to contribute to the pathogenesis of DS, although function of most of the encoded proteins still remains unknown.

DSCR contains genes coding for enzymes such as superoxide dismutase 1 (SOD1), cystathione beta synthase (CBS), glycinamide ribonucleotide synthase - aminoimidazole ribonucleotide synthase - glycinamide formyl

transferase (GARS-AIRS-GART). The function of SOD1 is to remove harmful peroxide linkage. In fetal DS brain, an increase in SOD1 activity has been observed, which may cause oxidative stress as well as gestational lipoperoxidation(2). Increased CBS activity may disrupt homocysteine metabolism, since in vitro studies have shown that increased CBS activity ultimately lead to folate trap(7). Over expression of Gars-Airs-Gart gene has been reported in fetal DS brain(2). Additionally, elevated blood levels of uric acid, xanthine and hypoxanthine, catabolites of purine metabolism, have also been observed in DS babies(2).

Down syndrome cell adhesion molecule gene (Dscam) at 21q22.2-22.3 is expressed in heart during cardiac development and is implicated in DS related congenital heart disease(8). Altered expression of collagen VI alpha 1, located at 21q22.3, may lead to abnormal neuronal migration since collagen controls cell proliferation and neuronal differentiation(9).

Modified expressions of genes for several neuro-signalling molecules have also been noticed in DS patients. Increased S100 beta, important for synaptogenesis and dendritic development(9), is detected in the brain and blood of subjects with DS. Over-expression of S100 beta molecules during fetal brain development could be an important cause of mental retardation(9). Over-expression of mini brain gene (Mnb) in fetal DS brain may impair learning and memory function(2). Increase in amount of some of the receptor molecules like GluR5 in DS brain may be involved in neuronal damage and disruption of neural differentiation(2). Over expression of Down syndrome critical region-1 (Dscr-1) gene could block dephosphorylation, nuclear translocation and activity of NFAT, a transcription factor, thereby inhibiting

calcineurin-dependent signaling(9,10). Over expression of Ets2, another transcription factor, have also been observed which may contribute to the increase in rate of neuronal apoptosis in DS(11).

Increase in dosage of amyloid precursor protein (App) gene at 21q21.3-22.05, may generate characteristic senile plaques and neurofibrillary tangles in the brain of almost all 40 years old DS patient(12). Further, increase in amyloid beta production could also be induced by over-expression of BACE2 at 21q22.3 during fetal development and in adult DS patients with Alzheimer disease (AD)(12).

### **Murine models**

The 21q22 region in human contains ~ 55 genes that are syntenic with mouse chromosome 16, 17 and 10(13). Experimental transgenic and trisomic murine models have been developed for investigating the molecular genetics of DS.

#### *Transgenic models*

Transgenic mouse models (*Table I*) have been designed to study the effect of cell-specific and stage-specific gene over-expression. These include genes for Copper Zinc superoxide dismutase 1 (CuZnSod1), neurotrophic factor S100 beta, beta amyloid peptide App, transcription factor Ets2, Drosophila minibrain homolog Dyrk1 and the transcription factor single minded 2 (Sim2). It was shown that transgenic mice containing human CuZnSod1 had 1.6 to 6 folds increased enzyme activity as compared to control(14), associated with decreased plasma serotonin levels and serotonin accumulation rate in transgenic mouse platelets(14), a phenomena similar to that reported in DS. Young TgS100-beta mice showed dendritic abnormalities similar to fetal DS brain(15). On the other hand, adult TgAPP mouse exhibited over-expression of APP in the neo-cortex and

**TABLE I**—List of Some Transgenic Mouse Model for DS.

Murine models	Human syntenic region	Features associated with different transgenic model
Tg SOD 1	<i>Sod 1</i> (21q22.1)	Abnormal neuromuscular junction decreased plasma serotonin level and increased biogenic amine function(14).
Tg S100 $\beta$	<i>S100 <math>\beta</math></i> (21q22.2-22.3)	Abnormal dendritic development(15).
Tg APP	<i>App</i> (21q21.3-22.05)	Dystrophic neuritis associated with congophilic plaques(16).
Tg Ets2	<i>Ets2</i> (21q22.3)	Skeletal abnormalities particularly craniofacial abnormalities(17) and brachycephaly(11).
Tg DYRK1	<i>Dyrk 1</i> (21q22.1)	Abnormal brain structure and locomotors behavior(18).
Tg mSim2	<i>Sim2</i> (21q22.2)	Learning deficiency(19).

hippocampus region mimicing features of DS(16). Over expression of Ets2 in TgEts2 resembled skeletal abnormalities(17) and neuronal apoptosis(11) of DS and that of *Dyrk1A* in the cerebellum of TgDyrk1a mice could be correlated with impairment in motor learning and organized locomotor behavior(18). TgSim2 is an ideal model for studying the Down syndrome associated mental retardation(19).

#### Trisomic models

Ts16 was the first trisomic model for DS with several phenotypic features similar to DS patients(20). Mouse chromosome 16 is about 35% larger to human chromosome 21 and therefore, mouse Ts16 has more extensive genetic imbalance than that of trisomy 21. Ts16 mice show delayed brain development in terms of cerebellar foliation, hippocampal fissure formation, immunologic and stem cell defects along with congenital heart disease(20). Short survival period (~14 days of gestation) is the main limitation of Ts16 trisomic model(20) and therefore, studies are confined to early developmental stages, cell culture systems and neuronal Ts16 tissue grafted into brain ventricles.

Two other mouse models, Ts65Dn(21) and Ts1Cje(22) result from partial trisomy of

mouse chromosome 16 and mimic many features of human trisomy 21. Ts65Dn mouse has longer survival (~36 months), genetic homology to the long-arm of human chromosome 21(21) and carries three copies of a region extending from app to mx1 (*Fig. 1*). This model shows developmental delay, skeletal malformation, decreased cerebellar volume and granular cell density, abnormal synaptic plasticity, abnormal pain responsiveness and sterility similar to DS patients, making this model an ideal experimental tool(21). Ts65Dn mice also show age related atrophy, neurodegeneration of basal forebrain cholinergic neurons and extensive astrocyte hypertrophy which resembles the neuropathology of AD in DS patients(23). Both APP mRNA and APP protein level are elevated in the cerebral cortex at embryonic day 15 and certain pathological changes like plaques and tangles appear in the Ts65Dn brain. In this context, Ts65Dn is also considered as a model for AD. Ts1Cje is trisomic for the sod1 to mx1 region(22), survive until adulthood and show several phenotypic features, behavioral abnormalities, reduced cerebellar volume and anomalies in craniofacial development like that of Ts65Dn(21). The Ts65Dn and Ts1Cje have been bred to develop MS1Ts65

### Key Messages

- Down syndrome (DS) is the most common genetic cause for mental retardation.
- Increased copy numbers of chromosome 21 (trisomy 21) accounts for ~95% of cases.
- Despite the identification of novel candidate genes, variable phenotypic changes associated with this syndrome still remains poorly explained.
- Different murine models have been developed to characterize the effect of genetic imbalance or dosage.
- Development of efficacious therapeutic regimes is dependent on improvement in screening, experimental models, as well as understanding of DS etiology.

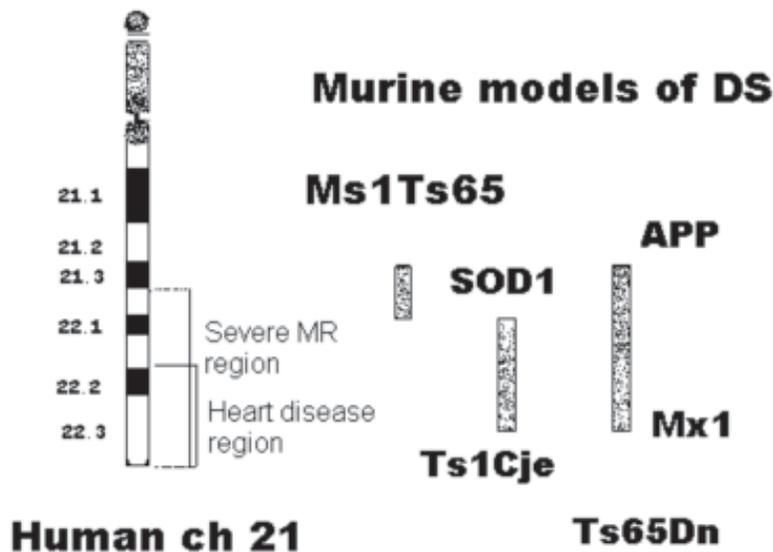


Fig. 1. Partial phenotypic map of human chromosome 21 and its syntenic regions in transgenic murine models.

(Fig. 1) mice that are trisomic for the segment spanning from *app* to *sod1* (24).

These models are very useful tools in identifying candidate genes and generating cDNA chips for investigating gene expression profile in trisomic mice versus DS patients. However, none of these murine models fully mirror features of DS, which suggests that other genes apart from those recognized as “candidate genes” also contribute to DS pathogenesis.

### Therapeutic approach

Down syndrome is a complex genetic disorder associated with various malformations along with mental retardation. In order to correlate phenotypic expressions with genotypes, the current experimental models need to be further developed. Extensive research involving improvement in the screening pattern and further refinement of model systems may lead to better understanding of the disease etiology and towards successful therapeutic management of the disorder.

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