Mental Retardation and Human Chromosome 21 Gene Overdosage: From Functional Genomics and Molecular Mechanisms Towards Prevention and Treatment of the Neuropathogenesis of Down Syndrome

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Abstract Down syndrome (DS), caused by a genomic imbalance of human chromosome 21 (HSA21), is mainly observed as trisomy 21 and is the major genetic cause of mental retardation (MR). MR and associated neurological and behavioural alterations result from dysregulation in critical HSA21 genes and associated molecular pathways. Gene expression, transcriptome, proteome and functional genomics studies, in human, trisomic and transgenic mouse models have shown similar genotype/phenotype correlation and parallel outcomes suggesting that the same evolutionarily conserved genetic programmes are perturbed by gene-dosage effects. The expression variations caused by this gene-dosage imbalance may firstly induce brain functional variations at cellular level, as primary phenotypes, and finally induce neuromorphological alterations and cognitive deficits as secondary phenotypes. The identification of trisomic genes overexpressed in the brain and their function, their developmental regulated expression and their downstream effects, their interaction with other proteins, and their involvement in regulatory and metabolic pathways is giving a clearer view of the origin of the MR in DS. This led to the identification of potential targets in the altered molecular pathways involved in MR pathogenesis, such as calcineurin, NFATs and MAPK pathways, that may be potentially corrected, in the perspective of new therapeutic approaches. Treatment of DS mouse models with NMDA receptor or $GABA_A$ antagonists allowed post-drug rescue of cognitive deficits. Besides these new pharmacotherapies, the regulation of gene expression by microRNAs or small interfering RNAs provide exciting possibilities for exogenous correction of the aberrant gene expression in DS and provide potential directions for clinical therapeutics of MR. Herein, we highlight the genetic networks and molecular mechanisms implicated in the pathogenesis of MR in DS and, thereafter, we outline some of the therapeutic strategies for the treatment of this as yet incurable cognitive disorder with a considerable impact on public health.

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Abbreviations

AChEI	Acetylcholinesterase inhibitor
AD	Alzheimer's disease
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
BAC	Bacterial artificial chromosome
BFCN	Basal forebrain cholinergic neurons
CA1	Cornu ammonis 1
CA3	Cornu ammonis 3
CaMKII	Calcium/calmodulin-dependent protein kinase
CBR1	Carbonyl reductase 1
ChAT	Choline acetyl transferase
CIT-K	Citron kinase
CREB	c-AMP response element-binding protein
DS	Down syndrome
DSCAM	Down syndrome cell adhesion molecule
DSCR	Down syndrome critical region
DSCR1	Down syndrome critical region gene 1
DYN1	Dynamin 1
DYRK1A	Dual-specificity tyrosine-(Y)-phosphorylation kinase 1A
EGF	Epidermal growth factor
EPSCs	Excitatory postsynaptic currents
ERG	Ets related gene
ES	Embryonic stem cells
ETS2	v-ets erythroblastosis virus E26 oncogene homolog 2
$GABA_A$	Gamma-aminobutyric acid type A receptor
GIRK2	G-protein coupled inward rectifying potassium channel subunit 2
HSA21	Human chromosome 21
IQ	Intelligence quotient
ITSN1	Intersectin gene 1
KCNJ6	Potassium inwardly rectifying channel J6
LPS	Lipopolysaccharide
LTD	Long-term depression
LTP	Long-term potentiation
MAPK	Mitogen activated protein kinase
MCIP1	Myocyte-enriched calcineurin-interacting protein 1
miRNA	MicroRNA

MMU16	Mouse chromosome 16
MR	Mental retardation
NFATc	Nuclear factor of activated T cells
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NMDA-R	N-methyl-D-aspartate receptor
PP1	Protein phosphatase 1
PTZ	Pentylenetetrazol
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RCAN1	Regulator of calcineurin 1 protein
S100B	S100 calcium-binding protein beta
SAGE	Serial analysis of gene expression
SHH	Sonic hedgehog
SIM2	Single minded 2
SNP	Single nucleotide polymorphism
SOD1	Superoxide dismutase 1
SYNJ1	Synaptojanin gene 1
TBS	Theta-burst stimulation
TPRD	Tetratricopeptide repeat domain Down syndrome
TTC3	Tetratricopeptide repeat domain 3
YAC	Yeast artificial chromosome

1 Mental Retardation in Down Syndrome: An Invalidating Neuropathological Aspect with Hard Impact on Public Health

Trisomy of human chromosome 21 (HSA21) is the most frequent genetic cause of mental retardation (MR) and other phenotypic abnormalities, including heart defects, cranio-facial abnormalities, cognitive impairment and Alzheimer's disease (AD), collectively known as Down syndrome (DS) and affecting 1 in 700 live births (Roizen & Patterson, 2003).

While the clinical phenotypes of each DS individual are variable in trait number and intensity, the MR remains the invariable hallmark disorder of DS and the most invalidating pathological aspect contributing to about 30% of all moderate-to-severe cases of MR (Lejeune, 1990; Pulsifer, 1996; Stoll, Alembik, Dott, & Roth, 1990).

Early infants show delayed cognitive development, leading to mild–moderate MR and decrease of the intelligence quotient (IQ) from early in the first year to late childhood (Brown, Greer, Aylward, & Hunt, 1990; Hodapp, Ewans, & Gray, 1999). DS patients have difficulties in both learning and memory. Moreover, the learning can be complicated by avoidance strategies when faced with cognitive challenges (Wishart, 1995). Although all domains of development follow the usual sequence, a deficiency in language production relative to other areas of development often causes substantial impairment (Chapman, Seung, Schwartz, & Kay-Raining Bird, 1998).

The impairment of prefrontal cortex and cerebellar function (Nadel, 2003), speech and articulation are also particularly affected. The lower performances of DS in linguistic tasks may be partially explained in terms of impairment of the frontocerebellar structures involved in articulation and verbal working memory (Fabbro, Alberti, Gagliardi, & Borgatti, 2002).

In adult life, the IQ of DS patients persists at low levels (30–70) and also undergoes a decline in cognitive performance (Chapman & Hesketh, 2000; Vicari, 2004, 2006) that has been interpreted as the consequence of accelerated ageing in DS (Devenny et al., 1996; Lott & Head, 2005). In addition, an early onset of an Alzheimer disease-like neurohistopathology is systematically observed by the fourth decade (Dalton & Crapper-McLachlan, 1986).

DS children have more behavioural and psychiatric problems than in other children, but fewer than in other individuals with MR. Adult DS patients can have a similar prevalence of psychiatric problems to other people with intellectual disability. A raised frequency of psychiatric problems is also related to the increased prevalence of depression in people with DS. However, they seem protected from some psychiatric disorders such as personality disorder, schizophrenia and anxiety (Collacot, Cooper, Branford, & McGrother, 1998). On the other hand, DS children show continuous but gradual improvement in mental age throughout childhood; IQs generally decline from early in the first year to late childhood (Hodapp & Zigler, 1990). Improvements in cognitive abilities and in quality of life of individuals with DS have resulted from improvements in medical care, identification and treatment of psychiatric disorders (such as depression, autism, and disruptive behaviour disorders) and early implementation of special educational programmes and interventions with typical educational settings (Connolly, Morgan, Russell, & Fulliton, 1993).

2 Mental Retardation in Down Syndrome: A Consequence of Developmental and Functional Brain Alterations

Individuals wih DS have a functionally abnormal brain with developmental alterations in morphogenesis and histogenesis. The brain of DS subjects is characterised by several postmortem macroscopic features that are related to pre- and post-natal abnormalities leading to retardation of brain growth (Schmidt-Sidor, Wisniewski, Shepard, & Sersen, 1990).

Infants and children with DS have delayed brain maturation, retardation of growth and delayed and disorganised second phase of cortical development and lamination emergence, cortical dysgenesis, delayed myelination, fewer neurons and lower neuronal density, abnormal synaptic connection (Wisniewski, 1990; Wisniewski & Schmidt-Sidor, 1989), shortened basilar dendrites, decreased number of spines with altered morphology, and defective cortical layering in several cortical areas (Becker, Armstrong, & Chan, 1986; Golden & Hyman, 1994; Marin-Padilla, 1976; Schmidt-Sidor et al., 1990; Takashima, Becker, Armstrong, & Chan, 1981; Takashima, Iida, Mito, & Arima, 1994).

Overall, brain volume is reduced in DS subjects (Becker, Mito, Takashima, & Onodera, 1991; Coyle, Oster-Granite, & Gearhart, 1986; Wisniewski, 1990), including cerebellar and cerebral grey and white matter (Kesslak, Nagata, Lott, & Nalcioglu, 1994; Pearlson et al., 1998; Pinter, Eliez, Schmitt, Capone, & Reiss, 2001; Raz et al., 1995; Schapiro, Haxby, & Grady, 1992; Schapiro, Luxenberg, Kaye, Haxby, & Friedland, 1989; Weis, Weber, Neuhold, & Rett, 1991). In particular, the reduced cerebellum shows a decreased volume of lobules VI to VIII (Ayraham, Sugarman, Rotshenker, & Groner, 1991; Jernigan, Bellugi, Sowell, Doherty, & Hesselink, 1993; Raz et al., 1995). Hippocampus volume is disproportionally reduced (Aylward et al., 1999; Kesslak et al., 1994; Krasuski, Alexander, Horwitz, Rapoport, & Schapiro, 2002; Pearlson et al., 1998; Pinter, Eliez et al., 2001; Raz et al., 1995), in particular at the level of the corpus callosum (Lai & Williams, 1989; Wang, Doherty, Hesselink, & Bellugi, 1992). The anterior cortex, including frontal and anterior temporal lobes, also appears reduced after adjustment for total cerebral grey matter volume (Jernigan et al., 1993; Lai & Williams, 1989; Teipel et al., 2004), whereas amygdala volume reductions do not exceed the overall reduction of brain size (Aylward et al., 1999; Pinter, Brown et al., 2001; Pinter, Eliez et al., 2001). On the other hand, an increased volume is found in other brain areas, such as ventricles (Ikeda & Arai, 2002; Kesslak et al., 1994; Pearlson et al., 1998; Schimmel, Hammerman, Bromiker, & Berger, 2006), parahippocampal gyrus after adjustment for overall brain volume (Kesslak et al., 1994; Raz et al., 1995; Teipel & Hampel, 2006; Teipel et al., 2003), temporal, parietal and posterior cortex, lenticular nucleus and thalamus and hypothalamus (Jernigan et al., 1993; Pinter, Eliez et al., 2001), while the occipital lobe and superior temporal gyrus do not show volume changes after adjustment for overall brain volume (Frangou et al., 1997; Pinter, Eliez et al., 2001). In addition, DS brains are characterised by several neurological defects in cortex lamination (Golden & Hyman, 1994) and in cerebellar foliation (Raz et al., 1995). Morphological and functional defects have also been found at the cellular level determined by alteration in neurogenesis, neuronal differentiation, myelination, dendritogenesis and synaptogenesis (Becker et al., 1986, 1991; Coyle et al., 1986; Dierssen & Ramakers, 2006; Huttenlocher, 1974; Marin-Padilla, 1972, 1976; Purpura, 1974; Takashima, Ieshima, Nakamura, & Becker, 1989; Takashima et al., 1994; Vuksic, Petanjek, Rasin, & Kostovic, 2002; Wisniewski, 1990; Wisniewski & Schmidt-Sidor, 1989).

Biochemical alterations also occur in foetal DS brain, which could serve as substrates for the morphological changes (Engidawork & Lubec, 2003), involving a decrease of the choline acetyltransferase and histidine decarboxylase activities and also a decrease of serotonin, histamine, and glutamate levels (Risser, Lubec, Cairns, & Herrera-Marschitz, 1997; Schneider et al., 1997; Wisniewski & Bobinski, 1991).

Taken as a whole, these alterations observed in the brain of DS, in particular those in the key regions involved in learning and memory processes, could be the origin of MR (Black, Nadel, & O'Keefe, 1977; Funahashi, Takeda, & Watanabe, 2004; Milner, Squire, & Kandel, 1998; Nadel & Willner, 1980). In addition, although young children with DS appear to be born with a normal septohippocampal cholinergic system (Kish et al., 1989), an ageing-dependent neurodegeneration of the basal forebrain cholinergic neurons (BFCN) was observed (Casanova, Walker, Whitehouse,

& Price, 1985; Yates et al., 1983). Because BFCN provide the major cholinergic input to the hippocampus and neocortex, the degeneration of these neurons may have functional consequences at the level of cholinergic receptors. These dysfunctions could produce additional learning and memory deficits in older individuals with DS (Yates et al., 1983) and could be an outgrowth of AD in these patients. In addition, an early onset of an Alzheimer disease-like neurohistopathology is systematically observed by the fourth decade (Dalton & Crapper-McLachlan, 1986).

The short- and long-term memory deficits observed in DS patients (Brown et al., 2003; Clark & Wilson, 2003; Hodapp et al., 1999; Hulme & Mackenzie, 1992) provide behavioural evidence of hippocampal dysfunction by adolescence (Carlesimo, Marotta, & Vicari, 1997). The spatial learning, also depending on the hippocampus, is particularly affected and there is also evidence for impairment of prefrontal cortex and cerebellar function (Nadel, 2003). In addition to the known effects of the hippocampal formation in spatial memory, the altered cortical layer and cerebellum also may participate to cognitive and behavioural phenotypes in DS (Funahashi et al., 2004).

Overall, the MR, the major neurological disorder of DS, is mainly a consequence of functional and developmental brain alterations in neurogenesis, neuronal differentiation, myelination, dendritogenesis and synaptogenesis.

3 Mental Retardation in Down Syndrome: A Consequence of Chromosome 21 Gene Overdosage

In the most cases, DS results from the trisomy of the HSA21 in all cells of the afflicted individuals (LeJeune, Gautier, & Turpin, 1959) and the generally accepted molecular origin of DS is the chromosomal imbalance associated to the HSA21 triplication and thus the overdosage of HSA21 genes that could be responsible for the phenotype seen in DS patients (Antonarakis, 1998).

In some rare cases, no more than 1% of living trisomic patients, DS results from a partial trisomy 21 showing variable phenotypes depending of the extra copy of the triplicated region. Clinical, cytogenetic and molecular analysis of such patients allowed narrowing a region of HSA21, called Down syndrome critical region (DSCR), localised on the distal part of the long arm, around the marker D21S55, and flanked by D21S17 and Ets related gene (ERG) (Delabar et al., 1993; Korenberg et al., 1994; Rahmani et al., 1989). The chromosomal imbalance due to the extra copy of DSCR is associated with the expression of many features of the disease and contributes significantly, but not exclusively, to MR (Delabar et al., 1993; Korenberg et al., 1994; Rahmani et al., 1989).

3.1 Chromosomal Imbalance Effects on Mental Retardation

The DSCR, containing the genes located between the carbonyl reductase 1 (CBR1) and the transcriptional regulator Ets-related gene (ERG) loci (Fig. 1) has been

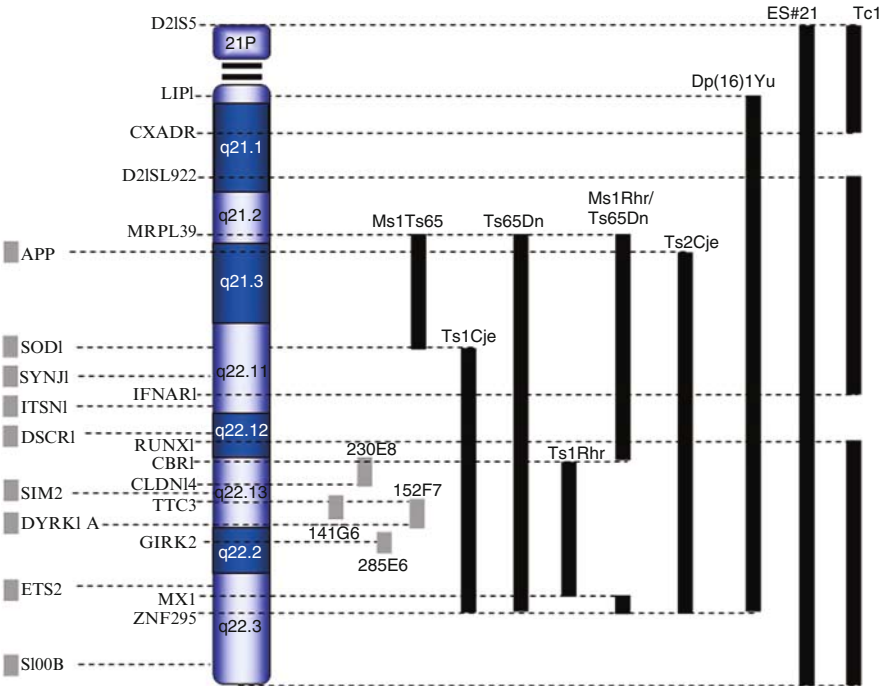

Fig. 1 Down syndrome mouse models. The boundary localisations of the HSA21 or its mouse syntenic triplicated regions in the DS mouse models are indicated on the right of the HSA21. *Black lines* indicate partial trisomic 16 and transchromosomal mice. *Grey lines* indicate segmental transgenic mice carrying human YACs, containing the DS critical region (DSCR). Monogenic transgenic mice are indicated on the left of the HSA21

designated as the DS critical region that, when duplicated, is associated with multiple neurological features of DS, including MR (Delabar et al., 1993; Korenberg et al., 1994; Toyoda et al., 2002). Consequently, the major phenotypes of DS, particularly MR, may have their origin in the over-dosage of genes located in DSCR. To explain the pathogenesis of DS from the genetic over-dosage, two genetic hypotheses have been considered.

3.1.1 Dosage-Sensitive Gene Hypothesis

This genetic hypothesis holds that the phenotype is a direct result of the cumulative effects of the dosage imbalance of the individual genes located on the triplicated HSA21 or critical region DSCR (Epstein, 1986, 1990; Korenberg et al., 1990). According to this "dosage-sensitive gene" hypothesis, a subset of genes on the triplicated HSA21 is directly responsible for particular pathological traits associated with trisomy 21. Consequently, the DSCR was defined as a minimal interval of the HSA21 that carries the dosage-sensitive genes necessary and sufficient for typical features of DS individuals (Delabar et al., 1993; Korenberg et al., 1994; Rahmani

et al., 1989). Several phenotypes of DS have also been found in transgenic mice engineered to overexpress HSA21 genes or their mouse orthologs. The observations of cardiac pathology, craniofacial dysmorphology, malformation of cerebellum and overall the deficit of cognitive functions both in DS individuals and in mouse models were in agreement with the "dosage-sensitive gene" hypothesis.

3.1.2 Amplified Developmental Instability Hypothesis

This genetic hypothesis, in contrast to the preceding hypothesis, states that dosage imbalance of the hundreds of genes on HSA21 determines a non-specific disturbance of genomic regulation and expression. This global disruption of the correct balance of gene expression in development pathways alters the normal developmental homeostasis and determines most manifestations of DS (Pritchard & Kola, 1999; Shapiro, 1983; Shapiro & Whither-Azmitia, 1997).

In agreement to this hypothesis, the variability of the DS phenotype in the different individuals has also been explained by intervention of stochastic factors during development (Kurnit, Aldridge, Matsuoka, & Matthysse, 1985), which can also be involved in normal development (Kurnit, Layton, & Matthysse, 1987). Moreover, several features observed in DS (for example, AD, cardiac malformations and metabolic diseases) can be observed in other trisomies and in the general population at lower frequency. In addition, the significant increase of the individual variability in DS, as compared to euploid individuals, also supports this hypothesis.

Nevertheless, the "amplified developmental instability" hypothesis and the "dosage-sensitive genes" hypothesis are not mutually exclusive. It is possible that single genes, or a specific subset of genes, may be involved in specific DS phenotypes, while some other DS phenotypes may be due to a more general disturbance in gene dosage imbalance as a result of the extra chromosomal material (Antonarakis, 2001).

3.2 Gene Dosage Imbalance in Down Syndrome Determine Dysregulation of HSA21 Gene Expression

3.2.1 Primary and Secondary Gene Effects

The genetic origin of DS is the overdosage of HSA21 genes that could be responsible for the genesis of the neurological and cognitive defects seen in DS patients (Antonarakis, 1998). The 1.5-fold increase of HAS21 gene dosage may determine a primary effect on gene transcription consisting in a 1.5-fold increase of expression level of these genes (Kurnit, 1979; Mao et al., 2005; Mao, Zielke, Zielke, & Pevsner, 2003).

Genes on the trisomic HSA21 encoding transcription factors and other proteins, that can directly or indirectly influence gene expression, may produce a secondary genome-wide transcriptional downstream misregulation, which may consist of

gene over-expression different from 1.5-fold, and also in down-regulation of genes on both HSA21 and the other chromosomes. This secondary effect of the chromosomal imbalance could be complex and highly variable in the different cells and during the lifespan (Dauphinot et al., 2005; Epstein, 1986, 1988; Lyle, Gehrig, Neergaard-Henrichsen, Deutsch, & Antonarakis, 2004; Prandini et al., 2007; Saran, Pletcher, Natale, Cheng, & Reeves, 2003; Sultan et al., 2007). An important application of the secondary gene effects on other chromosomes is widely used in antenatal screening programmes for trisomy 21 by detection of abnormal levels of foetal proteins in maternal serum. In particular, the level of the alpha fetoprotein is reduced (Newby et al., 1997) while human chorionic gonadotrophin is increased (Aitken et al., 1993) in trisomy 21, although the encoding genes are located on the chromosomes 4 and 19, respectively.

The molecular effects of the 1.5 gene overdosage may be even more complex at the protein level, as additional regulatory points are introduced, such as post-transcriptional, translational and post-translational regulations and post-translational modifications. Modification in the levels of proteins involved in multicomplex protein formation, in protein–protein intections and in metabolic and regulatory networks can determine alterations in these interactions because of the loss of the correct ratio among the proteins and, finally, can alter the function or stability of the proteins.

In the brain, it can be considered that the mere presence of the chromosomal imbalance determines misexpression and interaction of crucial genes/proteins involved in neuromorphogenesis and neurogenic cascades. The developmental errors caused by trisomy 21 during neural patterning and signal transduction pathways may lead to defective neuronal circuitry and could be the biological mechanism responsible for the pathogenesis of MR in DS.

3.2.2 Transcriptional Variation as a Consequence of Trisomy 21

DS may be considered as a multifactorial disease with an unusual aetiology characterised by overdosage of HSA21 genes determining gene expression variation that can be responsible for the complex DS phenotype. Thus, expression studies in normal and trisomic tissues contribute to understanding the role of the HSA21 genes and the contribution of their dosage alterations in DS pathogenesis, allowing the selection of HSA21 genes potentially involved in a given DS phenotype. In particular, a gene expressed in developing and/or adult brain may be selected as a candidate gene for MR, particularly when its transcription is restricted to key regions for cognitive functions, such as the hippocampal formation, the cortex and the cerebellum.

DNA sequencing of HSA21 (Hattori et al., 2000; International Human Genome Sequencing Consortium, 2004) and gene annotation improve identification of the gene products. These sequencing data and the development of molecular analysis tools allow large-scale application of gene expression analysis. Transcriptome studies are performed by quantitative RT-PCR (qRT-PCR), microarrays and serial

analysis of gene expression (SAGE), to study gene expression variation in trisomic tissues compared to the euploid ones using human tissues and cell lines (Aït Yahya-Graison et al., 2007; Chou et al., 2008; Deutsch et al., 2005; FitzPatrick et al., 2002; Giannone et al., 2004; Li et al., 2006; Malago et al., 2005; Mao et al., 2003, 2005; Prandini et al., 2007), or mouse trisomic model tissues (Amano et al., 2004; Chrast, Scott, Madani et al., 2000; Dauphinot et al., 2005; Kahlem et al., 2004; Lyle et al., 2004; Potier et al., 2006; Saran et al., 2003; Sultan et al., 2007; Wang et al., 2004). Transcriptome studies of the brain, the cerebellum or neuronal cell lines are particularly abundant, reflecting the major interest in the understanding of the molecular mechanisms involved in MR pathogenesis in DS (Amano et al., 2004; Chrast, Scott, Madani et al., 2000; Dauphinot et al., 2005; Kahlem et al., 2004; Lyle et al., 2004; Mao et al., 2003, 2005; Potier et al., 2006; Saran et al., 2003; Sultan et al., 2007; Wang et al., 2004).

Theoretically, the supernumerary copy of HSA21 is expected to result in a 50% increase in the level of transcripts of all genes mapping to HSA21. Most of these works confirm that transcript levels are elevated about 1.5-fold for the majority of trisomic genes in human trisomic tissues and across a broad range of tissues of trisomic mouse models (Aït Yahya-Graison et al., 2007; Amano et al., 2004; Dauphinot et al., 2005; Epstein, 1986; FitzPatrick et al., 2002; Kahlem et al., 2004; Lyle et al., 2004; Mao et al., 2003, 2005; Potier et al., 2006; Prandini et al., 2007; Sultan et al., 2007; Wang et al., 2004). These results indicate that the triplicated genes are overexpressed in a dosage-dependent manner, supporting the hypothesis that a global HSA21 dosage imbalance causes the heterogeneous phenotypes of DS (Shapiro, 1983, 1997). It cannot be excluded that the overexpression of a limited number of genes on HSA21 is responsible for the DS phenotypic features (Korenberg et al., 1990).

In addition, in several studies, it was also found that there is not always a direct correlation between genomic imbalance and not all genes are overexpressed ~1.5-fold compared to euploid (Aït Yahya-Graison et al., 2007; Dauphinot et al., 2005; Kahlem et al., 2004; Lyle et al., 2004; Potier et al., 2006; Saran et al., 2003), and a decreased expression is also found for some genes, such as GRIK1 (Saran et al., 2003), Ets2 (Engidawork & Lubec, 2003; Greber-Platzer, Schatzmann-Turhani, Cairns, Balcz, & Lubec, 1999; Wang et al., 2004), superoxide dismutase 1 (SOD1) (Engidawork & Lubec, 2003; Wang et al., 2004), DSCR3 (Engidawork & Lubec, 2003), HMGN1 (Engidawork & Lubec, 2003), and CCT8 (Engidawork & Lubec, 2003). For these dysregulated genes, the authors suggested that the initial overexpression of genes from the aneuploid chromosome was amplified by subtle compensatory mechanisms to the gene-dosage effect that may, in turn, result in the extensive variability of the phenotype that characterises DS (Dauphinot et al., 2005; Kahlem et al., 2004; Lyle et al., 2004).

The euploid genes, or the euploid region of chromosome 16, in the case of the DS mouse models, were generally found differentially expressed (over or under) (Bahn et al., 2002; Chrast, Scott, Madani et al., 2000; Chrast, Scott, Papasavvas et al., 2000; Dauphinot et al., 2005; FitzPatrick et al., 2002; Mao et al., 2003, 2005; Potier et al., 2006; Saran et al., 2003). These data support a model of a subtle primary upregulation of genes on the trisomic chromosome resulting in a more generalised secondary transcriptional misregulation (FitzPatrick et al., 2002).

Examination of difference of gene expression in two independent experiments suggests that the global perturbation includes a significant stochastic component. Thus, dosage imbalance of 124 genes in Ts65Dn mice alters the expression of thousands of genes to create a variable trisomic transcriptome (Saran et al., 2003).

At the present time, few studies have been performed concerning the HSA21 genes that do not have mouse homologs on the mouse chromosome 16 (MMU16). When gene expression was examined in Ts43H mice, a segmental Ts17 mouse model for DS, 20 brain-specific genes at dosage imbalance gave an average of 1.2-fold increased expression of euploid, with expression of only two genes reaching 1.5-fold expression (Vacik et al., 2005). In addition, 12 genes on the nontrisomic portion of chromosome 17 had expression levels that were 90% of euploid level. Brains from Ts2Cje mice exhibited a 1.5-fold expression level of specific trisomic genes comparable to Ts65Dn and different from euploid. Further data and analyses in both humans and mice are needed to reach biologically significant conclusions (Antonarakis & Epstein, 2006; Reeves, 2006).

Recently, new aspects of gene expression have acquired more importance in DS studies. The level of the expression variation for a given gene can change in the different tissues, including brain (Dauphinot et al., 2005; Kahlem et al., 2004; Lyle et al., 2004; Mao et al., 2005; Rachidi, Lopes, Charron et al., 2005; Rachidi, Lopes, Delezoide, & Delabar, 2006; Rachidi et al., 2000; Saran et al., 2003; Sultan et al., 2007) and during developmental stages (Dauphinot et al., 2005; Potier et al., 2006; Rachidi, Lopes, Costantine, & Delabar, 2005; Rachidi et al., 2006; Rachidi et al., 2000; Sultan et al., 2007), indicated that there were tissue- and cell-specific changes of gene expression in trisomy 21 during foetal development.

Inter-individual gene expression variations can explain at least some phenotypic individual differences, including susceptibility to common disorders. Since the 1970s, quantitative differences in gene expression have been proposed to explain variation in natural populations, participating in evolution and contributing to phenotypic diversity (King & Wilson, 1975). Recent studies indicate that variation in gene expression levels within and among populations is abundant, with significant inter-individual variation (Brem, Yvert, Clinton, & Kruglyak, 2002; Cheung et al., 2003; Oleksiak, Churchill, & Crawford, 2002; Schadt et al., 2003). Most of the differentially expressed genes had significant heritability (Brem et al., 2002; Monks et al., 2004; Morley et al., 2004; Spielman et al., 2007; Storey et al., 2007; Yvert et al., 2003).

This inter-individual gene expression variation has also been observed for HSA21 genes in DS (Aït Yahya-Graison et al., 2007; Deutsch et al., 2005; FitzPatrick et al., 2002; Prandini et al., 2007; Stranger et al., 2005; Sultan et al., 2007), and significant eQTLs have been identified (Deutsch et al., 2005). In particular, a cis-eQTL was identified for CCT8 corresponding to a single nucleotide polymorphism (SNP) located within the cis-regulatory region of CCT8 (Deutsch et al., 2005). These results are in agreement with the hypothesis that a molecular mechanism for the variability of phenotypic manifestations of trisomy 21 is a threshold effect of expression of HSA21 genes that show variable levels of expression in the population (Antonarakis, Lyle, Dermitzakis, Reymond, & Deutsch, 2004).

On the basis of the observed extensive variation in gene expression observed among normal individuals, it has been predicted that for many HSA21 genes there is a considerable overlap in total expression levels between normal and trisomy 21 individuals due to allelic variation (Aït Yahya-Graison et al., 2007; Deutsch et al., 2005; Prandini et al., 2007; Sultan et al., 2007). In this way, two considerations can be proposed. First, the expression variations can explain the differences in the penetrance and variability of the DS phenotypes. It has been proposed that overexpressed genes, showing low levels of expression variation, would be predicted to lead to the more penetrant phenotypes. In contrast, genes with high variation in expression would contribute to incompletely penetrant/variable DS-related phenotypes (Aït Yahya-Graison et al., 2007; Deutsch et al., 2005; Prandini et al., 2007; Sultan et al., 2007). Second, the existence of expression variations suggest caution in the analyses of the gene expression changes, particularly for low variation ratios (less than twofold), because of overlapping of gene expression variation in DS and normal individuals in this variation interval (Aït Yahya-Graison et al., 2007; Deutsch et al., 2005; Prandini et al., 2007; Sultan et al., 2007). In this way, a recent work analysed inter-individual gene expression variations between HSA21 genes in trisomic and normal cell lines. When pooled RNAs were used, a global gene dosage-dependent expression of chromosome 21 genes was observed. In contrast, when inter-individual gene expression variations were analysed, most of the HSA21 genes results compensated for the gene-dosage effect (Aït Yahya-Graison et al., 2007). The authors suggested that overexpressed genes are likely to be involved in DS phenotypes, in contrast to the compensated genes. Moreover, a more recent work analysed the differences in euploid gene expression variation between trisomy 21 and euploid tissues, on the hypothesis that these differences may contribute to the phenotypic variations in DS (Chou et al., 2008). The authors found a group of euploid genes showing greater expression variance in human trisomy 21 tissues than in euploid tissues, and that the number of euploid genes with elevated variance was significantly higher in DS tissues than in the euploid tissues (Chou et al., 2008).

Recently, new transcripts, the microRNAs (miRNAs), have been identified that play a role in gene expression regulation. MiRNAs are small, non-protein coding RNAs that link specific mRNA targets and lead to translational repression or mRNA cleavage (Bartel, 2004; Bushati & Cohen, 2007; Wang, Stricker, Gou, & Liu, 2007). Moreover, miRNAs have been shown to play a fundamental role in diverse biological and pathological processes, including cell proliferation, differentiation, apoptosis, carcinogenesis, and cardiovascular disease (Bushati & Cohen, 2007; Wang et al., 2007). It has been demonstrated that each miRNA can potentially regulate a large number of protein-coding genes, and many miRNAs can act in combination to regulate the same target genes (Bushati & Cohen, 2007; Wang et al., 2007). Thus, miRNA target genes are not restricted to a particular functional category or biological pathway, but rather are involved in a wide variety of biological processes.

Very recently, bioinformatic analyses have demonstrated that HSA21 harbours five miRNA genes: miR-99a, let-7c, miR-125b-2, miR-155, and miR-802 (Kuhn

et al., 2008). HSA21 miRNA expression analyses demonstrate that they are overexpressed in foetal brain and heart specimens from individuals with DS when compared with controls (Kuhn et al., 2008; Sethupathy et al., 2007). Moreover, some miRNAs, located on chromosomes other than 21, have been found overexpressed or underexpressed in hippocampus specimens from individuals with DS when compared to controls (Kuhn et al., 2008). The overexpression of the five HSA21 miRNAs in DS individuals may result in the aberrant expression of a large number of proteins in a variety of tissues. Thus, their inhibition or knock-down should normalise the expression level of all miRNA/mRNA targets back to non-trisomic 21 levels. Very interestingly, these potentialities suggest that HSA21 miRNAs may provide novel therapeutic targets in the treatment of individuals with DS.

The application of the global genomic approach to in situ expression analysis allowed the establishment of expression atlas of the HSA21 genes for large gene screening and identification of candidate genes for DS phenotypes (Gitton et al., 2002; Reymond et al., 2002). Nevertheless, single gene approaches remain indispensable to determine precise gene expression map in different embryonic, foetal and adult ages in human and mouse. In addition, identification of the brain cell types expressing a given gene supplies fundamental information that helps gene function understanding (Lopes, Chettouh, Delabar, & Rachidi, 2003; Lopes, Rachidi, Gassanova, Sinet, & Delabar, 1999; Rachidi, Lopes, Charron et al., 2005; Rachidi et al., 2000, 2006). These spatio-temporal investigations have been particularly improved with a novel powerful microscopy technology, allowing in situ quantification of mRNA variations in different neuronal cell types in a given key structure of the brain (Rachidi, Lopes, Charron et al., 2005; Rachidi et al., 2000, 2006), and a novel quantitative method (quantitative assessment gene expression, QAGE) for assessment of in situ gene expression (Rachidi et al., unpublished data).

3.2.3 Proteomic Variation as a Consequence of Trisomy 21

It is known that quantity of proteins does always not correspond to the quantity of the corresponding mRNAs, because of several post-translational mechanisms that determine the final protein level in the cells in the given condition. Since the proteins are the final and functional products of the genes, to know how protein levels change in DS cells is a fundamental knowledge for understanding the real genotype/phenotype correlation and, finally, the DS pathogenesis.

Initially, western blots have been used to measure the expression level of individual proteins. Several studies, analysing individual or small number of proteins, identified several changes in protein levels. Between the proteins encoded on HSA21, collagen VI A1 chain, COL6A1 (Engidawork, Balic et al., 2001) was found decreased in DS tissues compared to the normal tissues, while HMG14 (Epstein, 2001), S100B (Griffin et al., 1998), carbonyl reductase (Balcz, Kirchner, Cairns, Fountoulakis, & Lubec, 2001), and synaptojanin (Arai, Ijuin, Takenawa, Becker, & Takashima, 2002) were found increased in DS tissues compared to the normal tissues. In addition, some proteins encoded on chromosomes other than

HSA21 also show level changes. In particular, EF1A1 and EF2 (Freidl, Gulesserian, Lubec, Fountoulakis, & Lubec, 2001), Adenosine triphosphate (ATP)-sensitive potassium channels (Kim & Lubec, 2001), synaptosomal associated protein 25 subunits, drebrin, nucleoside diphosphate kinase B, Rab GDP-dissociation inhibitor beta subunit, histidine triad nucleotide-binding protein (Weitzdoerfer et al., 2001), and stathmin (Cheon, Fountoulakis, Dierssen, Ferreres, & Lubec, 2001) were found decreased in DS tissues compared to the normal tissues, while alcohol dehydrogenase (Balcz et al., 2001) and nicotinic acetylcholine receptor beta 2 subunits (Engidawork, Gulesserian, Balic, Cairns, & Lubec, 2001) were found increased in DS tissues compared to the normal tissues.

In a serial study, Lubec et al. analysed expression levels of 31 proteins encoded on HSA21 (Cheon, Bajo, Kim et al., 2003; Cheon, Kim, Ovod et al., 2003; Cheon, Kim, Yaspo et al., 2003; Cheon, Shim, Kim, Hara, & Lubec, 2003; Ferrando-Miguel, Cheon, & Lubec, 2004) and only three proteins showed different expression levels in DS compared to controls: Hematopoietic adapter containing Src homology 3 (SH3) domain and sterile α motifs (HACS1) was decreased in DS, compared to controls (Cheon, Bajo, Kim et al., 2003), Synaptojanin-1 was increased in DS, compared to controls (Cheon, Kim, Ovod et al., 2003), and DSCR5 (PIG-P), a component of glycosylphosphatidylinositol-N-acetylglucosaminyltransferase (GPI-GnT) was overexpressed about twofold in DS, compared to controls (Ferrando-Miguel et al., 2004).

Genome-wide proteomic approaches are performed using 2D-gel electrophoresis that, more recently, was associated to mass spectrometry, quantifying the protein spots. One of the first works using a global approach, having a limited resolution power, identified 11 proteins, among the 49 proteins analysed, that are deregulated in cerebral cortex of foetal DS, none of which encoded on HSA21 (Engidawork, Gulesserian, Fountoulakis, & Lubec, 2003).

Using the same approach, Kadota et al. (2004) have used an in vitro neuronal differentiation system of mouse Embryonic stem (ES) cells containing a single HSA21 (TT2F/hChr21) (Shinohara et al., 2001), using TT2F parental ES cells as a control. The authors have detected only 18 proteins with significantly altered levels, including SOD1 and CCT8, which are encoded on HSA21 (Kadota et al., 2004). Among the other 16 proteins, they found matrix and structural proteins, heat shock/stress proteins, protein or translational regulators, nuclear transcriptional factors, and enzymes for energy and macromolecular metabolism (Kadota et al., 2004). Among these 16 proteins encoded on other human chromosomes, the authors identified 7 that were overexpressed: protein subunits Atp6v1a1 and Atp6v1b2 of the vacuolar ATPase proton pump, which mediate acidification of intracellular organelles for energy production and convention, actin- (T-plastin and Vil2), filament- (Krt2–8) and phospholipid- (Anxa4) related cytoskeleton proteins. In constrast, nine proteins were underexpressed significantly in TT2F/hChr21 cells compared with TT2F cells: AI850305, Eef1D and UchL1, involved in protein catabolism or translation regulation, heat shock proteins Hsp84-1, Hsp70 and Hsp86-1, microtubule- (Mapre2) and calmodulin- (Cnn3) related architectural proteins were underexpressed. Moreover, splicing regulatory elements, HnrnpF and HnrnpC, displayed

contradictory expression patterns of overexpression and underexpression, respectively (Kadota et al., 2004). Moreover, in a comparison between mRNA and protein level change in TT2F/hChr21 cells compared with TT2F cells, different features were identified. The expression of *Anxa4*, *Atp6v1a1*, *Atp6v1b2*, *Krt2–8*, *Vil2* (overexpressed), and of *HnrnpC*, *Mapre2*, *UchL1*, *AI850305* (underexpressed) showed consistent mRNA transcription and protein translation. In contrast, *Cnn3*, *Eef1D*, *Hsp70*, *Hsp84*, *Hsp86*, *HnrnpF* and *T-plastin* showed disagreement (Kadota et al., 2004), suggesting the existence of post-transcriptional regulation or translational modification.

Recently, Shin, Gulesserian, Verger, Delabar, and Lubec (2006) performed a proteomic approach using a non-mosaic polytransgenic mouse model for DS generated by inserting yeast artificial chromosomes (YACs), containing a fragment of the human critical region DSCR, into the murine genome (Smith et al., 1995). These mice carry 141G6 YAC and are polytransgenic for HSA21 genes DSCR3, 5, 6, 9, and tetratricopeptide repeat domain 3 (TTC3). The authors identified 45 proteins showing altered expression levels, among the 422 polypeptides, which were the products of 239 different genes, in mouse transgenic hippocampus compared to control, although none of DSCR3, 5, 6, 9, and TTC3 proteins was detectable using the low resolution Coomassie staining (Shin et al., 2006).

These aberrant protein expressions may lead to impairment of cognitive functions. In particular, calcium/calmodulin-dependent protein kinase (CaMKII) protein was decreased in the 141G6 mouse hippocampus (Shin et al., 2006), and it is known that alteration of the CaMKII-pathway leads to a downstream alteration of the c-AMP response element-binding protein (CREB) pathway associated with impairment of fear memory (Bourtchuladze et al., 1994). 141G6 mice showed a lower performance in fear conditioning against sound as acoustic conditional stimulus (Chabert et al., 2004) that could be explained by aberrant protein levels of CaMKII (Shin et al., 2006). In contrast, 141G6 mice showed no cognitive defect by using Morris water maze and the multiple T-maze paradigms (Chabert et al., 2004) that could be explained by threshold levels necessary to alter these functions that was not surpassed, or that these tests are not sensitive enough to detect minor cognitive alterations (Shin et al., 2006).

In recent years, it is emerging that protein alterations exist as polymorphisms among wild-type mice of different inbred strains. These polymorphic variations complicate the interpretation of the variation of protein level changes and their correlation to a given disease. Recently, Mao et al. (2007) conceived a simplified approach to analyse the effect of gene-dosage imbalance on proteome in a controlled environment by using mouse ES cells. They investigated four cell lines contained one single overexpressed gene (App, Snca, Dyrk1a, & Dopey2) and two cell lines with a duplication or a deletion, respectively, of a HSA21 segment containing 14 genes. The authors identified globally 255 proteins showing expression variation in the six cell lines. Four features appear in this study: (1) about the same numbers (70–110) of proteins showed expression alterations in each line, with dosage imbalance in only one or 14 genes; (2) dosage alteration of a single gene led to quantitative changes in a large number of proteins; (3) many proteins showed changed expression

levels in several cell lines (38 proteins have alteration in at least three lines); and (4) 114 proteins were altered only in one cell line (Mao et al., 2007).

On the basis of these observations, the authors proposed that the protein level changes may also be explained in part by a global response of the cellular proteome to the gene dosage defect, restoring the balance in the cellular proteome, on the hypothesis that quantitative changes of the proteome by gene dosage effects can be compensated by a rearrangement restoring a new balance. In this way, the cellular proteins were defined as balancer proteins, with altered quantity in several lines, and cell line-specific proteins, when altered only in one cell line. Balancer proteins would function as buffers in the proteome homeostasis without a direct functional correlation with the transgene(s) and among them, in contrast to the cell line-specific proteins, likely including proteins participating to common functional networks of the transgene(s) (Mao et al., 2007). Interestingly, the balancers have more non-synonymous SNPs in coding regions than cell line-specific proteins (Mao et al., 2007), indicating that balancers may have more tolerance towards quantitative changes, whereas cell line-specific proteins need more precise correlation between expression level and function.

4 Modelling Neuronal Alterations and Mental Retardation in Mouse Models of Down Syndrome

The alterations observed in brain of DS patients are likely to take place during embryogenesis and cannot be easily investigated at early stages of human development. Developmental studies in humans are extremely difficult and in vitro molecular biology and cell culture systems do not replicate the complex developmental processes perturbed by trisomy. These investigations became possible with the generation of the mouse models of DS, because of the ability to manipulate their genome genetically and the accessibility to all their tissues at different embryonic, foetal and adult stages.

Interestingly, a high degree of conservation of the genomes and molecular mechanisms exists between mouse and human, and human genes on chromosome 21 are syntenic to mouse genes on chromosome 16 (~26.5 Mb), chromosome 10 (~2.3 Mb) and chromosome 17 (~1.1 Mb) (Hattori et al., 2000; Mural et al., 2002; Toyoda et al., 2002). This genetic evidence between the two mammalian species supports the essential use of the mouse in animal models to study the disruption of the developmental process caused by trisomy.

More interestingly, the elevated gene expression due to trisomy is very comparable between mice and human and shows similar complexity and a comparable genetic effect with the same outcome on the features of the mouse analogous to DS phenotypes. This make the mouse models powerful tools for dissecting the phenotypic consequences of dosage imbalance that affect single genes or chromosome segments, and they have greatly enhanced our understanding of the cellular and biochemical mechanisms of gene dosage effects involved in DS.

4.1 Trisomic Mouse Models and Candidate Chromosomal Regions for Mental Retardation in Down Syndrome

These trisomic mouse models with segmental or complete trisomy for MMU16, containing the orthologous region of the most part of HSA21, imitate the genetic complexity seen in trisomy 21 and have clinical phenotypes that correspond well to that observed in DS patients (Table 1).

Table 1 Trisomic and transgenic (Tg) mouse models for Down syndrome, their human syntenic chromosomal regions and genes and their neurological alterations

Models	Human syntenic region and genes	Neurological alterations
Ts16	(21q21.1–21q22.3) Trisomic for LIP1–ZNF295 region: 158 genes	Decreased brain size; cellular hypoplasia; abnormal neuronal migration.
Ts65Dn	(21q21.2–21q22.3) Trisomic for MRPL39–ZNF295 region: 136 genes	Alterations in cerebellum, hippocampus, and cortex; synapses, neurotransmitters; BFCN, learning and memory deficits
Ts1Cje	(21q22.11–21q22.3) Trisomic for SOD1–ZNF295 region: 83 genes	Reduced cerebellar volume and some brain defects similar to Ts65Dn; learning deficits
Ms1Ts65	(21q21.2–21q21.3) Trisomic for MRPL39–SOD1 region: 53 genes	Spatial learning impairement even less severe than Ts65Dn and Ts1Cje
Ts1Rhr	(21q22.13–21q22.3) Trisomic for CBR1–MX1 region: 33 genes	Altered brain volume and shape
Ts2Cje	(21q21.3–21q22.3) Trisomic from APP- to ZNF295: 132 genes	Decreased spines density of dendrites; enlarged dendritic spines
Dp(16)1Yu	(21q21.1–21q22.3) Trisomic for LIP1–ZNF295 region: 158 genes.	Not identified
Tc1	(HSA21 with two gaps: Cxadr-D21S1922; Ifnar1-Runx1) Trisomic for 92% of HSA21 genes.	Altered cerebellar neuronal number, synaptic plasticity, learning and memory
ES#21	HSA21 chimaera, Trisomic for HSA21 genes	Impairment in behaviour and learning
Tg SOD1	SOD1 (21q22.11), superoxide dismutase, key enzyme in the metabolism of oxygen-derived free radicals	Decreased serotonin level; neuronal degeneration in brain; learning defects
Tg APP	APP (21q21.3), β-amyloid precursor protein in senile plaque formation in DS and AD	Dystrophic neuritis associated with involved congophilic plaques; learning defects
Tg Synj1	SYNJ1 (21q22.11), synaptojanin 1 polyphosphoinositide phosphatase in synapses	Learning and memory defects

(continued)

Table 1 (continued)

Models	Human syntenic region and genes	Neurological alterations
Tg Ets2	ETS2 (21q22.2), erythroblastosis virus E26 transformation-specific transcription factor	Neuonal cell apoptosis; brachycephaly; neurocranial and cervical skeletal defects,
Tg S100β	S100β (21q22.3), calcium-binding protein beta neurotrophic factor released by astrocytes	Abnormal dendritic development; astrocytosis; learning and memory deficits
Tg Dyrk1 TgYac152F7	DYRK1A (21q22.13),Cbr1-Cldn14 (21q22.12-q22.13) containing Dyrk1A dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	Abnormal brain structure; increased brain weight and neuronal size; learning deficits
Tg Sim2	SIM2 (21q22.13), single minded, transcription factor/helix-loop-helix, master regulator in CNS cell fate	Altered behaviour and learning deficits
Tg DSCR1	DSCR1 (21q22-12), Down syndrome critical region 1	Neurological phenotype; impaired working memory in null mice
TgYac230E8	TTC3-DYRK1A (21q22.13) containing DOPEY2	Increased cortical neuronal density; learning deficits

These mouse models represent powerful tools allowing a genetic dissection of the complex DS phenotype, identifying different candidate chromosomal regions, syntenic with HSA21, and candidate genes involved in mouse brain alterations, and permitting a study of the early developmental phenotypes and the molecular and cellular pathogenesis of the brain abnormalities and MR in DS.

4.1.1 Ts16 Mice: Trisomic for Most Part of HSA21 with Three Copies of Complete MMU16

Ts16 mice, the first trisomic model for DS (Epstein, 1986; Lacey-Casem & Oster-Granite, 1994), carry three full copies of MMU16, which contain a region orthologous to the larger part of the HSA21 (Cox, Smith, Epstein, & Epstein, 1984; Gearhart, Davisson, & Oster-Granite, 1986).

The lethality in utero observed in Ts16 mice, due to the presence of three copies of genomic regions syntenic to other human chromosomes, limited the studies to cell lines and foetal stages. Interestingly, Ts16 foetuses have a number of phenotypes similar to those seen in DS patients, including brain alterations (Behar & Colton, 2003; Cox et al., 1984; Epstein et al., 1985; Gearhart et al., 1986; Lacey-Casem & Oster-Granite, 1994).

4.1.2 Segmental Ts65Dn Mice: Trisomic for Most HSA21 Genes Conserved in Distal End of MMU16

The Ts65Dn is the first segmental trisomy model created and is the most frequently used and the greatest characterised mouse model (Davisson, Schmidt, & Akeson,

1990). Ts65Dn mice are trisomic for most of the HSA21 orthologous genes conserved in the distal end of MMU16, extending from the gene for mitochondrial ribosomal protein L39 (Mrpl39) to the Znf295 gene, at the distal telomere (Fig. 1). Moreover, Ts65Dn mice are also trisomic for a c. 6 Mb region of chromosome 17 not syntenic to HSA21 (Akeson et al., 2001; Li et al., 2007).

Ts65Dn mice display many features that are reminiscent of those seen in people with DS, particularly the neurological phenotypes, including learning and behavioural abnormalities (Sago et al., 1998, 2000). This mouse model shows delayed brain development, decreased cerebellar volume and granular cell density, decreased dentate gyrus, and abnormal synaptic plasticity (Baxter, Moran, Richtsmeier, Troncoso, & Reeves, 2000; Davisson et al., 1990; Kleschevnikov, Belichenko, Villar, Epstein, & Malenka, 2004). Ts65Dn mice also show age-related atrophy, neurodegeneration of BFCN, neurotransmitters alterations and extensive astrocyte hypertrophy, which resembles the neuropathology of AD in DS patients (Casanova et al., 1985; Cooper et al., 2001; Dierssen, Vallna, Baamonde, Garcia-Calatayud, & Lumbreras, 1997; Yates et al., 1983).

The abnormal learning and behavioural abnormalities, analogous to DS MR, have been demonstrated using different behavioural tests such as T-maze, Y-maze and radial maze. Ts65Dn mice also show important learning defects in the Morris water maze that is the most commonly used test for spatial learning in almost all DS mouse models, and in which the cognitive performances of the different segmental trisomy 16 mouse models can be compared.

In the hidden platform test, Ts65Dn mice must learn the spatial relationships between objects in the room and the position of the platform to escape from the water. Ts65Dn mice showed increased search time compared with control and impaired performance that is not improved over successive trials indicating poor learning. In the probe test, in which the platform has been removed, the mice had learned the location of the platform and should search where the platform had been located. In this test, which assesses spatial selectivity, Ts65Dn mice showed a greater preference for the trained quadrant than control mice, providing evidence for learning. However, Ts65Dn mice spent significantly less time in the trained quadrant and crossed the trained site significantly less frequently than did controls. In the reverse platform test, the mice are required to learn a novel position for the hidden platform that has been moved to the quadrant opposite to its original location. Ts65Dn mice showed no decrease in latency, spent more time in the initial trained quadrant and showed increased time to reach the novel position of the platform. In the reverse probe dwell test, Ts65Dn mice continue to show a preference for the initial trained site. In the reverse probe crossing test, Ts65Dn mice failed to show a preference for the trained site. In these different Morris water maze tests, Ts65Dn mice show significant learning and memory deficits with a severe impairment in spatial learning and reversal, but not in visual discrimination learning and reversal (Holtzman et al., 1996; Reeves et al., 1995; Sago et al., 2000).

Interestingly, the long-term potentiation (LTP) is reduced in the cornu ammonis (CA1) and dentate gyrus areas of the hippocampus in the Ts65Dn (Kleschevnikov et al., 2004; Siarey et al., 1999; Siarey, Stoll, Rapoport, & Galdzicki, 1997) and the excitatory and inhibitory inputs to pyramidal neurons in cornu ammonis (CA3) of

the hippocampus are reduced (Hanson, Blank, Valenzuela, Garner, & Madison, 2007). This is particularly interesting since the hippocampal LTP, a form of synaptic plasticity evoked by a train of electrical stimuli, is considered a physiological model of learning and memory.

4.1.3 Segmental Ts1Cje Mice: Trisomic for Three-Quarters of Genes of Ts65Dn, Including DSCR

The Ts(16C-tel)1Cje, or Ts1Cje, present a smaller extra segment of MMU16 than that of the Ts65Dn mice. This mouse model, generated by a fortuitous translocation during the targeting of Sod1 by homologous recombination, carries the translocation from the proximal break-point in Sod1, that is not functional, to Znf295 (Sago et al., 1998) (Fig. 1). The Ts1Cje mouse is trisomic for about three-quarters of the genes that are present in the Ts65Dn mouse.

Ts1Cje mice have similar phenotypes to Ts65Dn, often with lower intensity, and fewer similarities to DS than do Ts65Dn mice, but they are important to study the particular effects of trisomy for a subset of genes triplicated in Ts65Dn and not in Ts1Cje. In particular, the neurological phenotypes in Ts1Cje are similar to those observed in Ts65Dn, such as the reduced volume and granule cell of the cerebellum (Olson, Roper et al., 2004) and the reduced LTP in the CA1 and dentate gyrus areas of the hippocampus (Siarey, Villar, Epstein, & Galdzicki, 2005).

Ts1Cje mice also show behavioural abnormalities in the Morris water maze tests. These mice displayed moderate to severe impairment in the hidden platform and probe parts of the test. In the reverse platform test, they showed a decrease in latency over the trials of the test, but the rates of decrease were significantly less than the controls and they were not significantly better than Ts65Dn. There was no preference of the trained quadrant in the reverse probe dwell test and Ts1Cje did significantly better than Ts65Dn in reverse crossing and dwell tests (Sago et al., 1998, 2000).

Comparison of the behavioural performances of the Ts1Cje and Ts65Dn in the Morris water maze showed that, except in the reverse probe tests, the learning deficits of Ts1Cje mice are similar to those of Ts65Dn. These findings indicate that an important gene or genes involved in these deficits lie in the overlapping region in these mice, from Sod1 to Mx1, and containing the critical region DSCR.

4.1.4 Segmental Ms1Ts65 Mice: Trisomic for Non-DSCR Genes of Ts65Dn and Missing from Ts1Cje

Ts65Dn mice, produced by reciprocal translocation T(17;16)65Dn, and Ts1Cje mice, carrying the reciprocal translocation T(12;16)1Cje, have been mated to produce offspring called Ms1Cje/Ts65Dn, or Ms1Ts65, that are trisomic for the genes present in Ts65Dn and missing from Ts1Cje, corresponding to the segment from MRPL39 to SOD1 (Fig. 1) (Sago et al., 2000).

These segmental trisomic mice have fewer similarities to DS than do Ts65Dn and Ts1Cje mice. In Ms1Ts65 mice, a neurological alteration has been identified at the cerebellar level, in which the granule cell density is moderately reduced similarly to Ts1Cje compared to Ts65Dn mice, which show significant reduction (Baxter et al., 2000; Olson, Roper et al., 2004).

The spatial learning and memory performances of Ms1Ts65 mice, tested by the Morris water maze (Sago et al., 2000), showed reduced latencies in the hidden platform test. In the probe test, the performance of Ms1Ts65 mice was similar to the controls. In the reverse platform test, mice such as Ts1Cje and Ms1Ts65showed a decrease in latency, but the rates of decrease were significantly less than the controls. Although the difference in latency between Ms1Ts65 and Ts1Cje was not statistically significant, Ms1Ts65 was significantly better than Ts65Dn whereas Ts1Cje was not. In the reverse probe dwell test, Ms1Ts65 was also significantly better than Ts65Dn in reverse crossing and dwell tests (Sago et al., 2000). Compared with controls, Ms1Ts65 mice show significant deficits in the latencies of the hidden and reverse hidden platform tests, but not in the probe tests. These results indicate that Ms1Ts65 has little impairment in learning the task in the Morris water maze compared with controls, while their deficits are significantly less severe than those of Ts65Dn. Therefore, whereas triplication of the region from Sod1 to Mx1 plays a major role in the abnormalities of Ts65Dn in the Morris water maze, triplication of the region from App to Sod1 also contributes to the poor performance.

4.1.5 Segmental Ts1Rhr and Ms1Rhr Mice: Trisomic and Monosomic for DSCR

A duplication, Dp(16Cbr1-Mx1)1Rhr, or Ts1Rhr, and a deletion, Ms1Rhr (Fig. 1), of the MMU16 segment between *Cbr1* and *Mx1* genes have been created using Cre-loxP and ES cell technologies (Olson, Richtsmeier, Leszl, & Reeves, 2004). These mice provide trisomy and monosomy, respectively, for a smaller segment of MMU16 that is orthologous to the critical region DSCR of HSA21 responsible for many of the features of the DS, including craniofacial abnormalities and MR.

Ts1Rhr mice have less severe craniofacial dysmorphology than either Ts1Cje or Ts65Dn (Olson, Richtsmeier et al., 2004). Both Ts1Rhr and Ms1Rhr mice show changes in volume and shape of both cerebrum and cerebellum, but different from each other and from Ts65Dn mice (Aldridge, Reeves, Olson, & Richtsmeier, 2007). In contrast, the performances of these two mouse models in the Morris water maze were similar to euploid mice (Olson et al., 2007).

4.1.6 Segmental Ts2Cje Mice: Trisomic from APP to the Telomere

Ts{Rb[12.17(16)]}2Cje mice, or Ts2Cje (Fig. 1), carry a chromosomal rearrangement of the Ts65Dn genome whereby the marker chromosome has been translocated to chromosome 12 forming a Robertsonian chromosome. This stable rearrangement

confers fertility in males and increases the frequency of transmitted segmental trisomy through the female germline. Like Ts65Dn mice, Ts2Cje mice are about 20% smaller in size postnatally compared with euploid control littermates, and this smaller size persists throughout life (Villar et al., 2005).

This trisomic model retains a dosage imbalance of HSA21 homologous genes from *App* to the telomere and expression levels similar to Ts65Dn within the triplicated region. Similarly to Ts65Dn mice, significant decreases in the density on the dendritic spine of dentate granule cell neurons and enlarged dendritic spines are observed in the Ts2Cje mice (Villar et al., 2005).

Ts2Cje mice exhibit neurologica1 features comparable with those of Ts65Dn mice, thereby validating the utility of this segmental trisomy model for the study of the molecular, genetic and developmental mechanisms underlying DS.

4.1.7 Segmental Dp(16)1Yu Mice: Trisomic from LIP1 to ZNF295

To generate a more complete trisomic mouse model of DS, a duplication has been established recently spanning the entire HSA21 syntenic region on MMU16 in mice using Cre/loxP-mediated long-range chromosome engineering. This new DS mouse model carries a chromosomal duplication, Dp(16)1Yu (Fig. 1), spanning 22.9 Mb of the complete HSA21 syntenic region 21q11q22.3 of the MMU16, delimited by the mouse orthologs of LIP1 and ZNF295 genes (Li et al., 2007).

The analysis of several genes located within Dp(16)1Yu in the brain and heart tissues showed that the segmental trisomy altered the transcript levels of the genes in the brain and heart of the Dp(16)1Yu/+ model, reflecting the dosage imbalance for the duplicated region. This result supports the conclusion that the duplicated genes are expressed with the exception for transcriptionally inactive genes.

About 37% of Dp(16)1Yu/+ embryos exhibit structural heart defects, and about 26 and 22% of Dp(16)1Yu/+ embryos exhibit annular pancreas and malrotation of the intestine, respectively. These phenotypes are also observed in patients with DS at higher frequencies than normal individuals. The cardiovascular and gastrointestinal phenotypes of the mouse model were similar to those of patients with DS. This new mouse model is particularly interesting because of the largest duplication of the HSA21 syntenic region and its stability, and it represents a powerful tool to further understand the molecular and cellular mechanisms of DS.

4.1.8 Transchromosomal ES(#21) Mice: Trisomic for a Large Part of HSA21

To maximally mimic the DS phenotypes, transchromosomal mouse models, carrying a HSA21 or a large part of it, have been generated (Fig. 1). These mouse models, containing an additional entire or partial HSA21, have been developed using a microcell-mediated chromosome transfer approach (MMCT) (Shinohara et al., 2001). The initial transchromosomic mouse model was obtained by transferring a

HSA21 into mouse ES cells. ES cell lines retaining HSA21 as an independent chromosome were used to produce chimeric mice with a substantial contribution from HSA21-containing cells. Chimeric mice derived from these cells, named ES(#21), in which a high percentage of cells contained a HSA21, demonstrated specific parallels to developmental anomalies seen in DS and a wide range of behavioural abnormalities indicating abnormal brain development and function. Interestingly, these mice present similar phenotypes to those observed in DS, such as thymus and cardiac defects, impairment in learning or emotional behaviour found in open-field, contextual conditioning and forced swim (Shinohara et al., 2001).

The high correlation between retention of HSA21 in the brain and behavioural and cognitive alterations found in these transchromosomal mice make them good models to study the complex and critical aspects of DS phenotype, because they provide the complete set of genes that are in dosage imbalance in human with trisomy 21. Further, these genes are introduced in mice into the context of their native cis-acting regulatory elements and chromatin structures; this maximises temporal and tissue-specific gene expression and function under physiologically appropriate conditions.

4.1.9 Transchromosomal TC1 Mice: Trisomic for Almost HSA21 (92% of HSA21 Genes)

Another transchromosomal mouse model, Tc1, has been generated containing an almost complete HSA21 with only two deletions. This mouse model represents the most complete animal model for DS currently available and carrying 92% of human genes (Fig. 1) (O'Doherty et al., 2005).

Tc1 mice showed alterations in cerebellar neuronal number, in heart development, and in mandible size. In addition, they have impaired short-term recognition memory and display reduced LTP in the dentate gyrus of the hippocampus, as well as showing a deficit in a novel-object recognition task (O'Doherty et al., 2005). Thus, Tc1 display many aspects of human DS but also recapitulate several of the DS features present in other mouse models (Reeves, 2006). Tc1 mice also have impaired spatial working memory but preserved long-term spatial reference memory in the Morris water maze (Morice et al., 2008). These mice showed a loss of the HSA21 from about 50% of the cells in adult mice, determining a high degree of mosaicism. The effect of this mosaicism may be different in the individuals and contributes to the variability of the phenotype. Therefore, unlike other segmental DS mouse models, Tc1 mice are also trisomic for orthologous genes on mouse chromosomes 10 and 17, consisting of a condition more similar to the trisomy 21 in human (O'Doherty et al., 2005).

4.1.10 Segmental Transgenic Mouse In Vivo Library of Human DSCR

The identification of the critical region DSCR and its association to MR suggests that this major and invariable DS trait arises from triplication of one or few genes

located in the DSCR. Interestingly, several genes located in the DSCR are involved in brain development and function and the overexpression of these critical genes determine cognitive alterations.

In vivo libraries of large insert transgenic mice offer an approach to study the contribution of a genomic region to complex quantitative traits. These mice are frequently transgenic for many genes and, thus, it is possible to investigate the cumulative effects of these genes upon one biological phenotype at a time, allowing multiplex analysis of the relationship between genotype and phenotype. Phenotypic and functional analysis of the in vivo library members could be used to define candidate genes for further analysis in human populations enabling association rather than linkage studies (Risch and Merikangas, 1996) to be employed in the identification of genes contributing to complex traits such as the MR in DS.

In this way, transgenic mice containing large fragments of the DSCR have been constructed (Smith et al., 1997; Smith, Zhu, Zhang, Cheng, & Rubin, 1995). The human genome fragments are 4 YACs, 230E8, 152F7 141G6 and 285E6, spanning 2 Mb of the DSCR (Dufresne-Zacharia et al., 1994; Smith et al., 1995). This panel of YAC transgenic mice propagating targeted megabase regions of the genome constitutes an in vivo library allowing genotype/phenotype comparison studies (Fig. 1).

The transgenic lines, carrying the YAC 152F7, containing six genes including TTC3 and dual-specificity tyrosine-(Y)-phosphorylation kinase 1A (DYRK1A) genes, show an increase of brain size and neuronal sizes (Branchi et al., 2004; Rachidi et al., 2007), and exhibit severe spatial learning and memory defects (Smith et al., 1997). The transgenic lines, carrying the YAC 230E8, containing seven genes of the DSCR region, including DOPEY2 gene, present increased cortical cell density (Rachidi, Lopes, Costantine et al., 2005; Smith et al., 1997), and increased length of the anterior lobules of the cerebellar vermis (Rachidi et al., 2007), and exhibit spatial learning and memory defects (Smith et al., 1997). The 141G6 mice showed a lower performance in fear-conditioning against sound as acoustic conditional stimulus (Chabert et al., 2004), although any evident neuroanatomical and cognitive defects have not yet been demonstrated in the 141G6 mice (Smith et al., 1997). Finally, the performances of transgenic lines carrying YACs 285E6 are not significantly different from the controls, and no detectable neurological defects have been found (Smith et al., 1997).

It is of greatest interest to dissect the role of these critical genes of DSCR, by separate analysis and study of their different combinations, to better understand the function of each gene or cooperation of gene groups, in neurological alterations and in learning and memory processes.

5 Genetic Dissection of the Role of the Down Syndrome Critical Region in Mental Retardation

In human, although the concept of the involvement of the critical region DSCR in the principal phenotypes of DS is largely accepted, its delimitation is not completely defined (Delabar et al., 1993; Korenberg et al., 1994; Rahmani et al., 1989), and its existence was rarely controversial (Shapiro & Whither-Azmitia, 1997).

The genotype/phenotype comparison approach has been applied to the mouse, and different mouse models have been generated allowing the evaluation of the role of the DSCR in DS pathogenesis, particularly in the MR. In a first approach, a transgenic mouse in vivo library has been developed, as described above, by inserting human YACs bearing different fragments of the human DSCR into the murine genome (Smith et al., 1995). The neuroanatomical alterations and defects in learning and memory observed in particular in two transgenic lines (152F7 and 230E8 YAC transgenic mice) indicate that these HSA21 fragments of the DSCR are critical for brain alterations and learning and memory defects, and that the correct dosage of critical genes of these DSCR fragments are crucial for brain function and cognitive impairment (Rachidi et al., 2007; Rachidi, Lopes, Costantine et al., 2005; Smith et al., 1997), in agreement with an important role of the critical region DSCR in neuronal and cognitive alterations observed in DS patients.

In an other approach, three other mouse models have been generated: Ts1Rhr mice trisomic for DSCR; Ms1Rhr mice with deleted DSCR; and Ms1Rhr/Ts65Dn mice obtained by breeding Ms1Rhr with Ts65Dn mice, trisomic for genes triplicated in Ts65Dn but not in the DSCR (Olson, Richtsmeier et al., 2004; Olson et al., 2007). Initially, the authors tested the association of craniofacial phenotypes to DSCR and found that three copies of DSCR alone are not sufficient to generate these phenotypes. Moreover, reducing trisomy of the DSCR to disomy in the Ts65Dn mice did not eliminate this phenotype, indicating that the DSCR is also not necessary to generate the cranio-facial phenotypes in mice (Olson, Richtsmeier et al., 2004). Recently, these studies have been extended to test the role of the DSCR in hippocampal function, learning and memory (Olson et al., 2007). Unlike Ts65Dn and Ts1Cje mice, no LTP impairment is detected in the CA1 hippocampal area of Ts1Rhr mice, consistent with the normal spatial learning in the Morris water maze showed by these mice. Thus, trisomy for DSCR is not sufficient to produce deficits in this hippocampal-based task (Olson et al., 2007). Ms1Rhr/Ts65Dn mice, with disomic DSCR, show identical performances to euploid in the Morris water maze. This indicates that the restoration of disomy of DSCR in trisomic mice rescues the spatial learning and memory performance, demonstrating that trisomy of DSCR is necessary for this cognitive phenotype (Olson et al., 2007). Thus, in contrast to the craniofacial phenotype, the combination of the behavioural results of Ts65Dn, Ts1Rhr and Ms1Rhr/Ts65Dn mice show that DSCR is necessary although not sufficient to determine the hippocampal dysfunction seen in Ts65Dn mice (Olson et al., 2007).

6 Transgenic Mouse Models of Down Syndrome

Contrary to segmental trisomic mice imitating the genetic complexity seen in trisomy 21 with eventual interactions between different genes present at three copies, the transgenic mouse models overexpress one or a few genes and allow a direct genotype/phenotype correlation.

The other interesting kinds of mouse models of DS are the transgenic monogenic mouse models that have been generated to study the effect of cell-specific and

stage-specific overexpression of a unique gene. These mice (Table 1; Fig. 1) include models for overexpression of the Cu-Zn superoxide dismutase1 (*SOD1* gene), the neurotrophic factor (*S100B* gene), the beta amyloid peptide (*APP* gene), the transcription factor (*ETS2* gene), the *Drosophila* minibrain homolog (*DYRK1A* gene), and the transcription factor single minded (*SIM2* gene), the regulator of calcineurin (RCAN1 or DSCR1 gene), the C21orf5 or (DOPEY2 gene), the tetratricopeptide repeat domain 3 (or tetratricopeptide repeat domain Down syndrome [TPRD] gene), the potassium inwardly rectifying channel (KCJN6 gene), the inersectin (ITSN1 gene), and the synaptoganin (SYNJ1 gene).

These transgenic mice showed overexpression of some genes in the key brain regions that play crucial roles in cognitive functions and that were found altered in the brain of DS patients. Moreover, for most of these genes, mouse models overexpressing them have an impaired behaviours and cognitive defects.

These transgenic mouse models allow the dissecting of the phenotypic consequences of imbalances that affect single genes and have greatly enhanced our understanding of the cellular and biochemical mechanisms of gene dosage effects involved in the developmental brain alterations and in the MR in DS.

7 Candidate Genes and Genotype/Phenotype Correlation for Mental Retardation in Down Syndrome

The final goal of genetic dissection is the identification of the gene(s) responsible of each phenotypic trait in DS. To date, several HSA21 genes have been identified as candidates for neurological alterations and MR in DS (Table 2), on the basis of different criteria. All these candidate genes show a strong expression in the key brain regions that play crucial roles in cognitive functions and that were found altered in the brain of DS patients. They are overexpressed in the brain of DS patients and/or in DS mouse models. Moreover, for most of these genes, mouse models overexpressing them have impaired behaviours and cognitive defects, similar to those observed in DS patients. Consequently, studies of these candidate genes and of the effects of their overexpression may help the understanding of the developmental brain alterations and the MR in DS.

7.1 Cu-Zn Superoxide Dismutase (SOD1) Gene

SOD1 gene encodes the Cu-Zn superoxide dismutase, a key enzyme in the metabolism of oxygen-derived free radicals. SOD1 product levels, both mRNA and protein, are increased in human and mouse trisomic tissues (Epstein et al., 1987; Kadota et al., 2004; Lyle et al., 2004; Mao et al., 2005; Saran et al., 2003).

Mice lacking SOD1 develop subtle motor symptoms by approximately 6 months of age, in which motor unit numbers are reduced early but decline slowly with age, suggesting that axonal sprouting are functionally impaired in the absence of SOD1 (Shefner et al., 1999).

Table 2 HSA21 genes over-expressed in Down syndrome brain and candidates for mental retardation

Genes	Brain regions	References
APP (amyloide beta A4)	Cortex, midbrain cerebellum	Epstein (2001), Saran et al. (2003), Lyle et al. (2004), and Kahlem et al. (2004)
SOD1 (superoxide dismutase 1)	Cortex, midbrain cerebellum	Saran et al. (2003), Lyle et al. (2004), Kadota et al. (2004), and Mao et al. (2005)
SYNJ1 (synaptojanin 1)	Cortex, midbrain cerebellum	Arai et al. (2002) and Lyle et al. (2004)
ITSN1 (intersectin 1)	Cortex, cerebellum	Pucharcos et al. (1999), Amano et al. (2004), and Lyle et al. (2004)
DSCR1 (calcipressin 1)	Cortex, midbrain cerebellum	Fuentes et al. (2000), Amano et al. (2004), Lyle et al. (2004), and Dauphinot et al. (2005)
DOPEY2 (DOPEY2/C21orf5)	Cortex, cerebrum	Lopes et al. (2003), Lyle et al. (2004), and Rachidi, Lopes, Costantine et al. (2005)
SIM2 (single-minded 2)	Midbrain	Vialard et al. (2000) and Lyle et al. (2004)
TTC3 (tetratricopeptide repeat domain 3)	Cerebrum, cerebellum	Saran et al. (2003), Amano et al. (2004), and Lyle et al. (2004)
DYRK1A (dual-specificity tyrosine-(Y)-phosphorylation kinase)	Cortex, midbrain cerebellum	Saran et al. (2003) and Lyle et al. (2004)
KCNJ6 (potassium inwardly-rectifying channel J6)	Cortex, midbrain	Saran et al. (2003), Lyle et al. (2004), and Kahlem et al. (2004)
Ets2 (v-ets erythroblastosis virus E26)	Cortex, cerebrum, cerebellum	Saran et al. (2003), Lyle et al. (2004), and Dauphinot et al. (2005)
DSCAM (Down syndrome cell adhesion molecule)	Cortex, midbrain cerebellum	Saran et al. (2003), Lyle et al. (2004), and Kahlem et al. (2004)
S100β (S100 calcium-binding protein β)	Cortex	Griffin et al. (1998) and Epstein (2001)

The transgenic mice containing human SOD1 had 1.6–6-fold increased enzyme activity as compared to control, associated with decreased plasma serotonin levels and serotonin accumulation rate in transgenic mouse platelets (Epstein et al., 1987), a phenomenon similar to that reported in DS. Human SOD1 transgenic mice show impairment in the ability to adjust their posture in response to a moving surface and show mild deficits in sensori-motor responsiveness (Lalonde, Dumont, Paly, London, & Strazielle, 2004; Lalonde, Le Pecheur, Strazielle, & London, 2005). The overexpression of Sod1 in transgenic mice leads to an impairment in LTP (Gahtan, Auerbach, Groner, & Segal, 1998) and defects in distal motor neuron terminals, indicating that this gene can selectively affect motor neurons (Avraham, Sugarman, Rotshenker, & Groner, 1991; Gurney et al., 1994). Moreover, these transgenic mice showed decreased cell number in several brain areas and decreased LTP in the pyramidal neuron CA1 (Harris-Cerruti et al., 2004; Zang et al., 2004).

Premature ageing, one of the characteristics of DS that contributes to decreased cognitive performance in DS adults, may involve oxidative stress and impairment of proteasome activity. Transgenic mice overexpressing the human SOD1 gene show a reduction in proteasome activities in the cortex and an associated increase in the content of oxidised SOD1 protein (Le Pecheur et al., 2005). These results suggest a role of this gene in development of axons and motor neurons.

7.2 Amyloid Precursor Protein (APP) Gene

Amyloide precursor protein (APP) gene encodes the beta-amyloid precursor protein, a protein involved in senile plaque formation in DS and AD (Kang et al., 1987). APP is widely expressed in axons, dendrites, and synapses in both central and peripheral nervous systems. In DS and Ts65Dn, APP is expressed at more than the expected 1.5-fold (Epstein, 2001; Hunter et al., 2003; Kahlem et al., 2004; Lyle et al., 2004), suggesting that other genes on HSA21 directly or indirectly can further up-regulate the APP gene.

The transgenic mice TgAPP exhibited overexpression of APP in the neocortex and hippocampus region mimicking features of DS. These amyloid precurseur protein transgenic models with AD-like pathology showed dystrophic neuritis associated with congopholic plaques (Sturchler-Pierrat et al., 1997) and also showed learning defects (Lamb et al., 1993). APP transgenic mice have been tested in the Morris water maze tests and they show impairment in the probe test, measuring the reference memory, and impaired performance in the reverse probe test, measuring the spatial working memory (Janus, 2004).

APP-null mice show impairment in the formation of LTP in the CA1 hippocampal region, and paired-pulse depression of GABA-mediated inhibitory post-synaptic currents is also attenuated, indicating that the impaired synaptic plasticity in APP deficient mice is associated with abnormal neuronal morphology and synaptic function within the hippocampus (Seabrook et al., 1999). Hippocampal neurons lacking APP show significantly enhanced amplitudes of evoked AMPA- and N-methyl-D-aspartate

(NMDA)-receptor-mediated excitatory postsynaptic currents (EPSCs), and increased size of the readily releasable synaptic vesicle pool, indicating that lack of APP increases the number of functional synapses (Priller et al., 2006). These findings suggest a role of APP in the neurophysiology of AD and DS.

7.3 v-ets Erythroblastosis Virus E26 (ETS2) Gene

The HSA21 protooncogene ETS2 encodes a transcription factor ETS2 (Watson et al., 1985), and alteration of its expression has been implicated in the pathophysiological features of DS.

ETS2 is expressed in neurons and is crucial for the normal formation of the neuromuscular junction (de Kerchove et al., 2002), and ETS2 is overexpressed in trisomic tissues (Dauphinot et al., 2005; Lyle et al., 2004; Saran et al., 2003).

Null mice homozygous for mutation of ETS2 are embryonic lethal and show trophoblast alteration (Yamamoto et al., 1998). Transgenic mice overexpressing ETS2 develop neurocranial and cervical skeletal abnormalities (Sumarsono et al., 1996), similarly to trisomy 16 mice and DS patients. The overexpression of ETS2 induces neuronal apoptosis, suggesting that overexpression of ETS2 may contribute to the increased rate of apoptosis of neurons in DS (Wolvetang, Bradfield, Hatzistavrou et al., 2003).

It has been found that ETS2 protein transactivates APP gene and that fibroblasts overexpressing ETS2 show molecular abnormalities seen in DS such as elevated expression of APP gene and increased beta-amyloid proteins (Wolvetang, Bradfield, Tymms et al., 2003). These findings suggest that ETS2 overexpression in DS determines overexpression of APP and may play a role in the pathogenesis of the brain abnormalities in Alzheimer disease and DS.

7.4 S100 Calcium Binding Protein B (S100B) Gene

S100B is a calcium-binding protein synthesised and released by astrocytes in response to serotonin (5-HT)-mediated stimulation of 5-HT1A receptors, and is an important extracellular neurotrophic agent during normal foetal brain development, with effects on neuroblasts and glia, involving the neuronal cytoskeleton (Azmitia, Griffin, Marshak, Van Eldik, & Whitaker-Azmitia, 1992; Morii et al., 1991).

It has been found that S100b null mice develop normally, with no evident alterations in the cytoarchitecture of the brain. However, they have enhanced LTP in the hippocampal CA1 region and also enhanced spatial memory in the Morris water maze tests and fear memory in the contextual fear conditioning. These results indicate that S100b is a glial modulator of neuronal synaptic plasticity and of information processing in the brain (Nishiyama, Knopfel, Endo, & Itohara, 2002).

The S100B RNA and protein are overexpressed in DS brain and Alzheimer disease (Epstein, 2001; Griffin et al., 1998). Transgenic mice overexpressing mouse

S100β or human S100B show changes in cytoskeletal markers, such as the dendritic-associated protein, MAP-2, the growth-associated protein-43 and the dendritic spine marker, drebrin, leading to an increased density of dendrites within the hippocampus (Shapiro and Whitaker-Azmitia, 2004). Interestingly, drebrin protein is decreased in DS and AD brain regions (Kojima & Shirao, 2007; Shin & Lubec, 2002). Alterations have also been found in astrocyte morphology and axonal sprouting, especially in the dentate gyrus of the S100B transgenic mice (Bell, Shokrian, Potenzieri, & Whitaker-Azmitia, 2003; Reeves et al., 1994; Shapiro & Whitaker-Azmitia, 2004). Mice overexpressing S100B show decreased spatial learning and memory in the Morris water maze, radial-arm maze and Y-maze (Bell et al., 2003; Gerlai & Roder, 1996; Whitaker-Azmitia et al., 1997; Winocur, Roder, & Lobaugh, 2001). These results suggest that S100B overexpression contributes to glial-neuronal interactions, dendritic abnormalities and MR in DS.

7.5 Dual-Specificity Tyrosine Y Kinase 1 Subunit A (DYRK1A) Gene

DYRK1A has been initially identified as the human homolog of the *Drosophila* minibrain gene, MNB, and is involved in neuroblast proliferation and reduction of the adult *Drosophila* brain (Tejedor et al., 1995). DYRK1A encodes a serine-threonine kinase (Kentrup et al., 1996). DYRK1A is expressed in the cortex, hippocampus and cerebellum (Guimera, Casas, Estivill, & Pritchard, 1999; Guimera et al., 1996; Rahmani, Lopes, Rachidi, & Delabar, 1998) and is overexpressed in mouse trisomic model Ts65Dn (Guimera et al., 1999), in DS foetal brain and in other trisomic tissues (Lyle et al., 2004; Saran et al., 2003).

The *Dyrk1A* mutant mice are lethal during gestation. The heterozygote mice (*Dyrk1A*$^{+/-}$) show a decreased size in several brain regions, a decreased neuronal cell number in the superior colliculus, an increased neuronal density in the cortex and in the thalamus, and exhibit neurobehavioural delays and defects (Fotaki et al., 2002). At cellular level, *Dyrk1A*$^{+/-}$ mice show smaller size of the pyramidal cell somata, shorter dendritic length, lower spine number, and altered spine distribution, suggesting the implication of *Dyrk1A* in the capability of the pyramidal cells to integrate information (Benavides-Piccione et al., 2005).

Two transgenic mouse models overexpressing DYRK1A have been generated. The first one carried a human YAC 152F7, containing DYRK1A (Smith et al., 1995), while the second carried the full-length DYRK1A cDNA (Altafaj et al., 2001). In the Morris water test, the transgenic lines carrying the YAC 152F7 showed lower performance in the probe test, in which the platform is removed. In the reverse learning paradigm, the transgenic mice showed the most severe deficits with no significant learning of the new platform position, indicating deficits in learning flexibility (Smith et al., 1997). A mouse line carrying a 152F7 YAC fragment (152F7tel) containing only the DYRK1A gene showed the same phenotype to the original YAC lines demonstrating that the overexpression of DYRK1A

is responsible for the learning and memory defects in these mice (Smith et al., 1997). Moreover, these transgenic mice showed increased brain size and neuronal size (Branchi et al., 2004; Rachidi et al., 2007).

The significant impairment in spatial learning and memory observed in the two mouse models overexpressing DYRK1A indicates that the correct dosage of DYRK1A gene is crucial for brain hippocampal and prefrontal cortex functions, particularly concerning a cognitive dysfunction of the reference memory (Altafaj et al., 2001; Smith et al., 1997). Moreover, the transgenic mice overexpressing Dyrk1A exhibit neurodevelopmental defects, delayed craniocaudal maturation and motor dysfunction (Altafaj et al., 2001; Fotaki et al., 2002). Recently, DYRK1A bacterial artificial chromosome (BAC) transgenic mice have also shown learning and memory defects (Ahn et al., 2006). In addition, these mice showed abnormal LTP and long-term depression (LTD), suggesting synaptic plasticity alteration (Ahn et al., 2006). These phenotypes are comparable with those found in murine models of DS with trisomy of chromosome 16, and suggest a causative role of DYRK1A in MR in DS patients.

Dyrk1A proteins are transported through the neuron dendrites and regulate their development (Hammerle et al., 2003), as also demonstrated by the overexpression of a kinase-deficient DYRK1A that impedes neurite outgrowth (Yang, Ahn, & Chung, 2001). Moreover, DYRK1A is co-localised in dendrites with Dynamin 1 (DYN1), a GTPase putative substrate of DYRK1A, involved in synaptic vesicle recycling, membrane trafficking and neurite outgrowth (Chen-Hwang, Chen, Elzinga, & Hwang, 2002; Hammerle et al., 2003). Dyrk1A proteins also modulate the activity of the CREB, which participates in signal transduction pathways involved in synaptic plasticity and neuronal differentiation (Hammerle et al., 2003). DYRK1A is involved in several pathways and it has recently been demonstrated that it can influence the NFATc pathways through its kinase activity (Arron et al., 2006; Gwack et al., 2006).

7.6 Single-Minded (SIM2) Gene

SIM2, the first gene identified in the DSCR region (Dahmane et al., 1995), shows a high homology with the *Drosophila* single minded gene, sim, encoding a transcription factor/helix-loop-helix protein (Crews, Thomas, & Goodman, 1988). The *Drosophila* sim is a master gene of the midline development in the central nervous system, functioning as transcriptional regulator in cell fate determination (Crews et al., 1988; Nambu, Lewis, Wharton, & Crews, 1991; Thomas, Crews, & Goodman, 1988).

The mammalian Sim2 is expressed in the embryonic brain in delimited regions of the neuroepithelium of D1 and D2 neuromeric regions and along the neural tube (Dahmane et al., 1995; Ema et al., 1996; Fan et al., 1996; Rachidi, Lopes, Charron et al., 2005). In later human foetal stages, SIM2 gene expression is found at different levels in discrete human brain regions, including the cortical layers, the hippocampus and the cerebellum (Rachidi, Lopes, Charron et al., 2005), which are key regions

involved in learning and memory, and are also altered in DS patients (Golden & Hyman, 1994; Ito, 2002; Milner et al., 1998; Miyashita, 2004; Raz et al., 1995).

Sim2 is overexpressed about 1.5-fold in Ts1Cje mouse foetuses (Vialard et al., 2000) and in trisomic tissues (Lyle et al., 2004). Transgenic mice overexpressing Sim2 display reduced sensitivity to pain and mild impairment of learning (Chrast, Scott, Papasavvas et al., 2000; Ema et al., 1999). These behavioural anomalies found in the Sim2 transgenic mice recall some phenotypes observed in trisomic mouse models for DS, Ts65Dn and Ts1Cje (Coussons-Read & Crnic, 1996; Martinez-Cue et al., 1999; Sago et al., 1998), and in DS patients (Hennequin, Morin, & Feine, 2000).

Sim2 mutant mice are lethal in the early post-natal days and show skeletal alteration due probably to cell proliferation defects (Goshu et al., 2002). Functional studies indicated that SIM2 protein control the Shh expression in the brain (Epstein et al., 2000), involved in cell growth and differentiation in the brain. Moreover, SIM2 can inhibit cell cycle by inhibition of cyclin E expression (Meng, Shi, Peng, Zou, & Zhang, 2006) suggesting a key role of SIM2 in neulogical alterations seen in DS.

7.7 Regulator of the Calcineurin (RCAN1) Gene

Also called Down syndrome critical region 1 gene (DSCR1) or myocyte-enriched calcineurin-interacting protein 1 (MCIP1) or calcipressin 1 (CSP1), the regulator of the calcineurin 1 protein (RCAN1) gene directly modulates the activity of the protein phosphatase, calcineurin. The CSP1, the protein encoded by DSCR1, interacts with calcineurin A (Fuentes et al., 2000) to inhibit calcineurin activity (Rothermel, Vega, & Williams, 2000).

DSCR1 is highly expressed in brain and heart (Fuentes et al., 1995). It is overexpressed in the brain of DS foetuses (Fuentes et al., 2000), in brains from DS patients with AD symptoms (Ermak, Morgan, & Davies, 2001), and in the brain of DS mouse models (Amano et al., 2004; Dauphinot et al., 2005; Lyle et al., 2004).

It has been observed that the calcineurin activity is decreased in AD (Ladner, Czech, Maurice, Lorens, & Lee, 1996), in DS foetal brain tissue, and in *Drosophila* mutants that overexpress DSCR1 (Chang, Shi, & Min, 2003). As DSCR1 is an inhibitor of calcineurin activity, it is possible that these changes could be caused by increased levels of DSCR1, as occurs in DS, promoting the development of AD. Indeed, overexpression of DSCR1 in rat primary neurones causes formation of aggresome-like inclusion bodies similar to those observed in DS and AD brains, as well as reducing the expression of the synaptic vesicle protein, synaptophysin, in neural processes (Ma et al., 2004).

Interestingly, loss-of-function and overexpression of mutants of nebula, the *Drosophila* orthologue of DSCR1, both display severe learning defects in several basic learning assays, indicating that DSCR1 may affect regulatory pathways of synaptic transmission (Chang et al., 2003). In agreement with this indication, forebrain-specific knock-out of calcineurin in mice results in impaired hippocampal-dependent memory tasks and synaptic plasticity (Zeng et al., 2001). DSCR1/Mcip1$^{-/-}$ mice have an impaired cardiac hypertrophic response to

pressure overload, suggesting that this gene may also function as a calcineurin facilitator in vivo (Vega et al., 2003). Mice deficient in Mcip1/2 show more dramatic impairment in cardiac hypertrophy than the DSCR1$^{-/-}$. Moreover, these DSCR1 knock-out mice displayed a neurological phenotype and showed faster overall movements in an open field test and a significant impairment in working memory, as assessed by novel and familiar object recognition analysis (Sanna et al., 2006).

Recently, DSCR1 has also been demonstrated to play a role in memory and synaptic plasticity by examining the behavioural and electrophysiological properties of DSCR1 knock-out mice (Hoeffer et al., 2007). These mice exhibit deficits in spatial learning and memory, reduced associative cued memory, and impaired late-phase long-term potentiation (L-LTP), phenotypes similar to those of transgenic mice with increased calcineurin activity. Consistent with this, the DSCR1 knock-out mice display increased enzymatic calcineurin activity, increased abundance of a cleaved calcineurin fragment, and decreased phosphorylation of the calcineurin substrate dopamine and cAMP-regulated phosphoprotein-32. These findings suggest that DSCR1 regulates LTP and memory via inhibition of phosphatase signalling (Hoeffer et al., 2007).

7.8 DOPEY2 Gene

C21orf5 gene, that we recently renamed DOPEY2 following HUGO nomenclature, is a member of the Dopey family containing leucine zipper-like domains involved in multiple protein–protein interactions (Rachidi, Lopes, Costantine et al., 2005).

DOPEY2 is more highly expressed in the differentiating zones than in the proliferating zones in embryonic human and mouse brain (Lopes et al., 2003; Rachidi et al., 2006), suggesting a role of DOPEY2 in cell differentiation and developmental patterning. This potential role is also supported by the high homology of DOPEY2 with the *Caenorhabditis elegans* Pad-1, required for embryonic patterning during gastrulation (Guipponi et al., 2000), the yeast Dop1 and DopA, required for normal growth patterning, and cell differentiation and organogenesis in fungi (Dujon, 1996; Pascon & Miller, 2000). DOPEY2 expression becomes restricted to cerebellum, cortex and medial temporal-lobe system during foetal development and in adult brain (Lopes et al., 2003; Rachidi et al., 2006), in which this gene shows different transcriptional intensities, as demonstrated by an improved new optic technology allowing comparison of the cell density and the expression intensity (Rachidi et al., 2006). These findings are of the most interest because the medial temporal-lobe system, including the hippocampal formation and perirhinal cortex, works as a control centre of the memory circuits and storage (Krasuski, 2002; Milner et al., 1998) and also, the cortex and the cerebellum participate in elaboration of memory (Ito, 2002; Miyashita, 2004). DOPEY2 is expressed in brain regions that play key roles in learning and memory and present neuronal alterations in DS patients (Golden & Hyman, 1994; Raz et al., 1995), suggesting a role of this gene in the learning and memory.

DOPEY2 is overexpressed in DS lymphoblasts 1.5–2-fold compared to normal lymphoblasts (Lopes et al., 2003) and in trisomic tissues (Lyle et al., 2004), suggesting that DOPEY2 is a dosage-sensitive gene. Transgenic mice carrying the human YAC 230E8 (Smith et al., 1995) contain the entire DOPEY2 gene (Lopes et al., 2003; Rachidi, Lopes, Costantine et al., 2005), and overexpress it at less than twofold (Lopes et al., 2003; Rachidi, Lopes, Costantine et al., 2005). These transgenic mice show increased cortical cell density (Rachidi, Lopes, Costantine et al., 2005; Smith et al., 1997) that overexpresses DOPEY2 (Rachidi, Lopes, Costantine et al., 2005). This phenotype corresponds well to the abnormal lamination pattern found in the cortex of DS patients (Golden & Hyman, 1994).

Recently, a new cerebellar phenotype has been discovered in two independent mouse lines carrying the YAC 230E8 characterised by elongation of the anteroposterior axis, increased length of rostral folia of the vermis, and abnormal culmen and declivus lobules (Rachidi et al., 2007). These phenotypes in the cortex and cerebellum may also explain the learning and memory deficits of these mouse models (Rachidi, Lopes, Costantine et al., 2005; Rachidi et al., 2007; Smith et al., 1997) suggesting a role of the DOPEY2 gene in neuropathological defects and MR in DS.

7.9 Potassium Inwardly Rectifying Channel (KCNJ6) Gene

Potassium inwardly rectifying channel J6 (KCNJ6) or G-protein coupled inward rectifying potassium channel subunit 2 (GIRK2) (Lesage et al., 1994; Ohira et al., 1997) encodes the GIRK2, a member of the ATP-sensitive potassium channels, involved in increase of the intracellular ATP concentration, linking cellular metabolism to the electrical excitability of the plasma membrane. GIRK2 is highly expressed in the brain, particularly in the cerebellar granule cell, suggesting a role of Girk2 in granule cell differentiation (Goldowitw & Smeyne, 1995). Girk2 mutation is responsible of the weaver phenotype in mouse, characterised by a drastically reduced cerebellum due to the depletion of granular cell neurons (Patil et al., 1995).

GIRK2 is overexpressed in trisomic tissues (Kahlem et al., 2004; Lyle et al., 2004; Saran et al., 2003) and in the brain of DS mouse model Ts65Dn, particularly in the hippocampus (Harashima, Jacobowitz, Witta et al., 2006), and determines an increase of GIRK channel density in Ts65Dn neurons and a twofold increase of $GABA_B$-mediated GIRK current (Best, Siarey, & Galdzicki, 2006). In addition, the GIRK2 overexpression seems to alter the GIRK1/GIRK2 ratio, which likely affects the balance between excitatory and inhibitory neuronal transmission in Ts65Dn, and thus overexpression of GIRK2 could contribute to DS neurophysiological phenotypes (Best et al., 2006; Harashima, Jacobowitz, Stoffel et al., 2006; Harashima, Jacobowitz, Witta et al., 2006).

Girk2 heterozygous animals show behavioural changes intermediate between wild-type and null mutants only in the elevated plus-maze test after social isolation (Harashima, Jacobowitz, Stoffel et al., 2006), in agreement with gene-dosage

dependence of RNA and protein expression (Blednow, Stoffel, Chang, & Harris, 2001). Girk2 homozygous mice show a higher level of locomotion and a higher number of rearing in the light area in the light/dark box. In the elevated plus-maze test, these mice spent a higher percentage of time in the open arms and showed a higher number of total entries. These results suggest hyperactivity and reduced anxiety in Girk2 null mice (Harashima, Jacobowitz, Stoffel et al., 2006).

7.10 Tetratricopeptide Down Syndrome (TPRD) Gene

TPRD, also called TTC3, gene (Ohira et al., 1996; Tsukahara, Hattori, Muraki, & Sakaki, 1996), is localised in the DSCR region. TPRD protein contains 2–3 units of a 34-amino acid repeat similar to the tetratricopeptide (TPR) motif, in the different splicing forms (Dahmane et al., 1998), that are involved in protein–protein interactions (Das, Cohen PW, & Barford, 1998; Groves & Barford, 1999).

TPRD shows regional and cellular specificity during mouse and human brain development, and its expression is higher in the differentiating areas than in the proliferating ones, suggesting a role of TPRD in neuronal cell differentiation (Lopes et al., 1999; Rachidi et al., 2000). The strong TPRD expression in the human foetal cortex corresponds to the crucial developmental stage when the size of the cortical mantle doubles in thickness and the cortical lamination begins, suggesting a role of TPRD in cortical lamination. Interestingly, TPRD shows a differential expression in the human foetal hippocampus and cerebellum with variable intensities in specific neuronal cell types as estimated by a novel microscopy technology allowing gene transcription quantification (Rachidi et al., 2000). This specific expression pattern corresponds well to abnormal brain regions seen in DS patients (Golden & Hyman, 1994; Raz et al., 1995). TPRD is overexpressed at more than 1.5-fold in trisomic tissues including brain (Amano et al., 2004; Lyle et al., 2004; Saran et al., 2003).

To date, two transgenic mice have been produced carrying human YACs, 141G6 and 152F7, each containing TPRD gene (Fig. 1) (Smith et al., 1997). The neurological and behavioural phenotypes observed in the 152F7 mice are determined by the DYRK1A gene overexpression. The 141G6 mice showed a lower performance in fear-conditioning against sound as acoustic conditional stimulus (Chabert et al., 2004), although any evident neuroanatomical and cognitive defects have not yet been demonstrated in the 141G6 mice (Smith et al., 1997). In addition, the 141G6 mice showed aberrant protein expression compared to control mice. Interestingly, these expression protein alterations may potentially lead to impairment of cognitive functions. In particular, these mice showed decreased CaMKII protein in hippocampus (Shin et al., 2006), and it is known that alteration of the CaMKII-pathway lead to a downstream alteration of the CREB pathway associated with impairment of fear memory (Bourtchuladze et al., 1994).

Recently, it has been demonstrated that TPRD/TTC3 protein interacts with citron kinase (CIT-K) and citron N (CIT-N), two effectors of the RhoA small

GTPase, involved in neuronal proliferation and differentiation (Berto et al., 2007). Interestingly, these authors demonstrated that TPRD/TTC3 overexpression strongly inhibits neurite extension, while its knock-down stimulates the neurite extension. In agreement with the previous results (Lopes et al., 1999; Rachidi et al., 2000), these dose-dependent effects are Rho-dependent, suggesting an important role of the TPRD-RhoA-CIT-K in neuronal differentiation (Berto et al., 2007). Overall, the expression pattern, the high overexpression of TPRD in the brain, and its role in neuronal differentiation suggest that this gene could be involved in fine neurological alterations in DS patients, and could participate to MR pathogenesis.

7.11 Down Syndrome Cell Adhesion Molecule (DSCAM) Gene

Down syndrome cell adhesion molecule (DSCAM), an axon guidance molecule mapping to HSA21 (Schmucker et al., 2000; Yamakawa et al., 1998), is expressed in the developing spinal cord and cortex. This gene may be involved in definition of the dorsal–ventral axis in the developing spinal cord at the time of neurite extension and may participate in the determination of regional neuronal fates in the developing cortex (Barlow, Micales, Chen, Lyons, & Korenberg, 2002).

DSCAM proteins are expressed in the cerebral and cerebellar white matter, in accordance with the temporal and spatial sequence of myelination. In DS brains, DSCAM protein level is increased in the Purkinje cells at all ages, and in the cortical neurons during adulthood, compared to that for controls. In demented DS patients, DSCAM protein appeared in the core and periphery of senile plaques. This DSCAM expression pattern suggests that this gene may play a role as an adhesion molecule regulating myelination. In addition, the overexpression of DSCAM may also play a role in the MR and the precocious dementia of DS patients (Saito et al., 2000).

The DSCAM *Drosophila melanogaster* homolog, dDscam, has been well characterised, and an important role has been demonstrated in neuronal wiring specificity (Chen et al., 2006; Hattori et al., 2007). During nervous system development, commissural axons project towards and across the ventral midline, a process mediated by netrin-1 and the netrin-1 receptor. It has been demonstrated recently that DSCAM is also required for commissural axon guidance. DSCAM is expressed on spinal commissural axons, binds netrin-1, and is necessary for commissural axons to grow towards and across the midline. Thus, overexpression of DSCAM, by causing enhanced netrin-DSCAM interactions, could contribute to the axonal wiring defects seen in DS (Ly et al., 2008).

7.12 Synaptoganin 1 (SYNJ1) Gene

Synaptojanin 1 (SYNJ1) is a polyphosphoinositide phosphatase that dephosphorylates the phosphatidylinositol-4,5-bisphosphate, a lipid that regulates membrane

transduction and membrane trafficking in the endocytic pathway at synapses. The phosphatidylinositol is a signalling phospholipid implicated in a wide variety of cellular functions. At synapses, where normal phosphatidylinositol balance is required for proper neurotransmission, the phosphoinositide phosphatase synaptojanin 1 is a key regulator of its metabolism.

Synaptojanin is highly enriched in the brain and is located at nerve terminals, and is associated with synaptic vesicles and coated endocytic intermediates (Haffner et al., 1997; McPherson, Takei, Schmid, & De Camilli, 1994). Moreover, the distribution of synaptojanin is coincident with that of other endocytic proteins, such as amphiphysin and dynamin (McPherson, Garcia, Slepnev, David, & Zhang, 1996; McPherson et al., 1994). For these reasons, synaptojanin may play a role in the endocytosis and could be involved in the recycling of synaptic vesicles.

Synaptojanin 1 mutant mice die early after birth and exhibit accumulation of clathrin-coated vesicles at nerve terminals and increased synaptic depression in hippocampus, supporting a role for synaptojanin in the uncoating step of the recycling pathway (Cremona et al., 1999).

The role that synaptojanin 1 plays in specific steps of the synaptic vesicle cycle has been studied by combined quantitative imaging with electron microscopy on cultured cortical neurons from synaptojanin 1 knock-out mice (Kim et al., 2002). The findings indicate that the rapid degradation of phosphatidylinositol by synaptojanin 1 is of critical importance for efficient synaptic vesicle regeneration and for the recovery of normal presynaptic function after an exocytic burst. In the absence of synaptojanin 1, sustained activity leads to a kinetic delay in synaptic vesicle reformation and to an increased, transient backup of synaptic membrane. This study provides direct evidence for the hypothesis that synaptojanin 1 plays a key physiologic role in the transition from early endocytic compartments to newly reformed synaptic vesicles fully incorporated into the functional pool. These results provide new evidence for a critical role of phosphoinositides in synaptic physiology and for their importance in regulating membrane traffic in the presynaptic terminal (Kim et al., 2002).

It has been found that phosphoinositide metabolism is altered in the brain of Ts65Dn mice and in transgenic mice overexpressing Synj1 from BAC constructs. This defect is rescued by restoring Synj1 to disomy in Ts65Dn mice. The Synj1 transgenic mice also exhibit deficits in performance of the Morris water maze task, suggesting that phosphoinositide dyshomeostasis caused by gene dosage imbalance for Synj1 may contribute to brain dysfunction and cognitive disabilities in DS (Vonorov et al., 2008).

7.13 Intersectin 1 (ITSN1) Gene

The human ITSN1 gene spans 250 kb of genomic DNA and maps to HSA21 (Pucharcos et al., 1999). ITSN1 protein has five consecutive SH3 domains (SH3A-E), commonly found in signal transduction and cytoskeletal proteins (Pawson, 1995),

and which interact with proline-rich domain-containing proteins involved in clathrin-mediated synaptic vesicle endocytosis (McPherson, 1999).

ITSN1 encodes two isoforms, a long and a short, and both are expressed in the brain, but the long form is neuronal-specific and the short form is expressed in glial cells (Hussain et al., 1999; Ma et al., 2003). In addition, the long isoform is expressed in zones of proliferating and differentiating neurones, in both adult and foetal mouse brain, and this long ITSN1 transcript is overexpressed in the brains of individuals with DS (Pucharcos et al., 1999).

ITSN1 seems to function as a scaffolding protein, providing a link between the components of endocytosis and the actin cytoskeleton, and has a role in signal transduction (Hussain et al., 1999; Jenna et al., 2002; Martina, Bonangelino, Aguilar, & Bonifacino, 2001; Roos & Kelly, 1998; Yamabhai et al., 1998). Interestingly, ITSN1 overexpression blocks clathrin-mediated endocytosis (Sengar, Wang, Bishay, Cohen, & Egan, 1999), presumably through disruption of higher order protein complexes between ITSN1 and its binding partners. Also, ITSN1 overexpression was found to block epidermal growth factor (EGF)-mediated MAPK activation by inhibiting Ras activation directly, most probably by preventing the Ras/mSos interaction, suggesting a role for ITSN1 in Ras activation (Tong et al., 2000).

Mice with a null mutation in Intersectin 1 (Itsn1) showed alterations in endocyic and vesicle trafficking, including reduced number of exocytosis events in chromaffin cells, slowing of endocytosis in neurons, increased endosome size in neurons and reduced nerve growth factor (NGF) levels and decreased levels of choline acetyl transferase (ChAT) positive cells in the septal region of the brain (Yu, Chu, Bowser, Keating, & Dubach, 2008). Interestingly, the presence of enlarged endosomes in the neurons of DS is an early sign of AD, suggesting that ITSN1 could contribute to the disturbance in endocytosis in early AD pathogenesis in DS (Yu et al., 2008).

7.14 Contribution of MicroRNAs in Down Syndrome Mental Retardation

MicroRNAs (miRNAs) are small, non-protein coding RNAs that base pair with specific mRNA targets leading to translational repression or mRNA cleavage (Bartel, 2004; Bushati & Cohen, 2007; Wang et al., 2007). MiRNAs are expressed as long primary transcripts that are subsequently processed into mature miRNAs (about 22 nucleotides) by several nuclear and cytoplasmic enzymatic steps (Bushati & Cohen, 2007; Wang et al., 2007). MiRNAs have been shown to play a fundamental role in diverse biological and pathological processes (Bushati & Cohen, 2007; Wang et al., 2007).

Recently, five miRNA genes harboured on HSA21, including miR-99a, let-7c, miR-125b-2, miR-155, and miR-802, have been identified (Kuhn et al., 2008). Among these miRNAs, the bic/miR-155 gene is well characterised and its expression is regulated by lipopolysaccharide (LPS) and cytokines (O'Connell, Taganov, Boldin, Cheng, & Baltimore, 2007; Taganov, Boldin, Chang, & Baltimore, 2006).

As described above, alteration of the expression of the five HSA21-derived miRNAs in DS has been studied, demonstrating that they are overexpressed in DS tissues when compared with normal tissues (Kuhn et al., 2008; Sethupathy et al., 2007). Moreover, other miRNAs have also been identified showing expression alteration (over- or downexpression) in DS tissues when compared with normal tissues (Kuhn et al., 2008).

These data suggest that trisomic 21 gene dosage overexpression of HSA21-derived miRNAs results in the decreased expression of specific target proteins and contributes, in part, to features of the neuronal and cardiac DS phenotype. Importantly, HSA21-derived miRNAs may provide novel therapeutic targets in the treatment of individuals with DS.

8 Potential Molecular Pathways and Mechanisms Involved in Mental Retardation of Down Syndrome

The important update of the genomic sequence and the identification of HSA21 genes, determination of candidate genes and their mouse orthologs for DS phenotypes, particularly those involved in brain alterations, learning and memory deficits, and also the development and improvement of public databases, datamining and algorithms to perform genome wide analyses (Gardiner, Fortna, Bechtel, & Davisson, 2003; Hattori et al., 2000; International Human Genome Sequencing Consortium, 2004; Kapranov et al., 2002; Nikolaienko, Nguyen, Crinc, Cios, & Gardiner, 2005), has produced important knowledge and tools to study the molecular effects of the expression variation of gene products from the triplicated genes and their functional variations predisposing to specific cognitive deficits in the goal to better understand the molecular pathophysiology of MR in the DS.

8.1 Molecular and Cellular Mechanisms Leading to Mental Retardation in Down Syndrome

Recently, we have proposed a global mechanism model explaining the molecular and cellular origin of MR in DS (Rachidi & Lopes, 2007). In this model, we considered the complexity of gene interactions allowing the gene expression variations caused by the gene overdosage. These expression variations may firstly induce functional alterations at cellular level in the brain, that we called the primary phenotypes, and the final combination of these neuronal alterations could determine brain morphological defects, behavioural alterations and the MR in DS, that we called the secondary phenotypes (Fig. 2).

We proposed that three principal genetic mechanisms could participate in concert to determine the final transcriptome and proteome alteration in DS. First, some

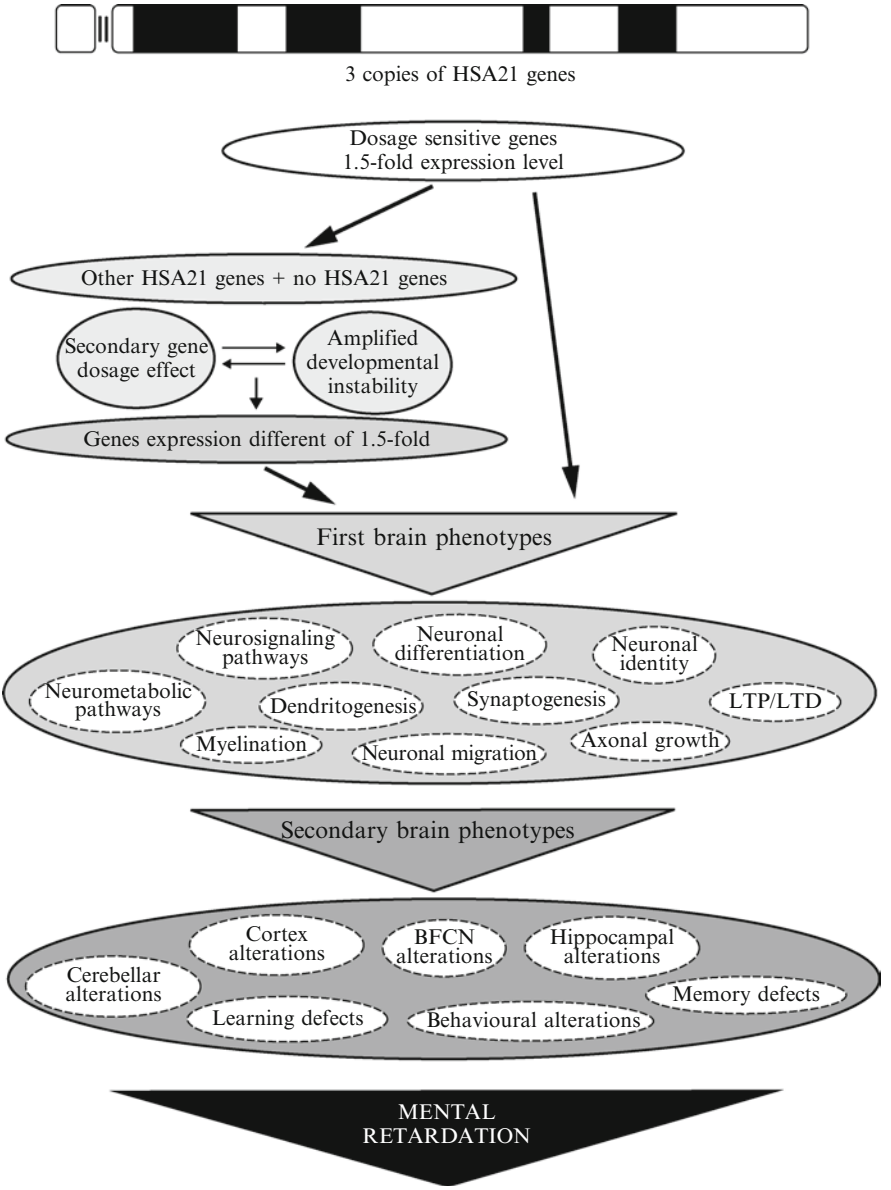

Fig. 2 Molecular and cellular mechanism leading to neurological phenotypes and mental retardation in Down syndrome. In this model, the overdosage of the HSA21 genes induces global alterations of gene expression via different genetic mechanisms. On the one hand, a primary effect of the gene overdosage determines a 1.5-fold gene overexpression. On the other hand, the overexpressed genes on the HSA21 could variably modify the expression level of other trisomic genes on HSA21 and of disomic genes on other chromosomes, by a secondary gene effect or by a more general alteration of the transcriptional homeostasis (amplified developemental instability). These gene expression variations may firstly induce functional alterations at cellular level in the brain, that we called the primary phenotypes. These neuronal alterations could determine the neuromorphological, neurological, and behavioural alterations, which we called the secondary phenotypes. The final combination of these neurological alterations leads to the mental retardation in DS

HSA21 genes, the dosage-dependent genes, are expressed on average 1.5-fold the normal level as a direct consequence of the 1.5 gene overdosage. In some cases, their overexpression could directly determine a primary phenotype in DS brain. Second, in addition, some of these dosage-dependent genes could modulate directly or directly the expression of target genes on both the HSA21 and the other chromosomes. In these cases, the modulation of the target gene expression could be different of 1.5-fold up-regulation and could also be down-regulated, depending on the nature of the dosage-dependent gene products, and thus determine a secondary gene dosage effect. And three, all or some HSA21 genes with altered expression could participate in the amplified developmental instability of the genome in trisomy 21, determining a more general gene expression alteration and disequilibrium (Fig. 2).

These gene expression changes in the brain determine alterations at cellular level, which we globally call primary phenotypes (Fig. 2), include metabolic pathways, regulatory cascades and cellular processes, such as proliferation, differentiation and apoptosis. Recently, alterations in synaptogenesis and dendritogenesis haver received increasing interest for their implication in MR. Abnormal ultrastructure and number of synapses and dendrites are observed both in DS patients and mouse models (Becker et al., 1986, 1991; Belichenko et al., 2004; Benavides-Piccione et al., 2004; Dierssen & Ramakers, 2006; Hanson et al., 2007; Kurt, Davies, Kidd, Dierssen, & Florez, 2000; Kurt, Kafa, Dierssen, & Davies, 2004; Takashima et al., 1994). Moreover, the synapses of trisomic brains also show functional alterations, such as LTP and LTD in the hippocampus and dentate gyrus (Kleschevnikov et al., 2004; Siarey et al., 1997, 1999, 2005), reduced noradrenergic function in the hippocampus (Dierssen et al., 1996, 1997), and reduced excitatory and inhibitory inputs to pyramidal neurons in CA3 of Ts65Dn hippocampus (Hanson et al., 2007).

These primary phenotypes and their combinations may determine the more complex secondary phenotypes, in particular functional alterations of the cognitive network and brain plasticity that participate in the MR in DS (Fig. 2). As an elucidated example, during cerebellar development in Ts65Dn mice, a primary cellular phenotype has been identified to be the molecular origin of a DS secondary neurological phenotype (Roper et al., 2006). In Ts65Dn mice, a cerebellar hypoplasia is observed, due to decreased cerebellar granular cells and their precursors. It has been demonstrated that reduced mitosis is determined by a deficit in response to the Sonic hedgehog (Shh) mitogenic signals (Roper et al., 2006). This suggests that a dosage-sensitive gene or genes make cells less sensitive to Shh when overexpressed, corresponding to the primary phenotype, and that determines the cerebellar hypoplasia corresponding to the secondary phenotype.

8.2 Molecular Pathways Contributing to Mental Retardation in Down Syndrome

The genetic disruption caused by trisomy 21 in neural patterning and signal transduction pathways during development leads to alteration of the neuronal circuitry

and could be the biological mechanism responsible for the pathogenesis of MR in DS. Thus, it is of the most interest to elucidate the molecular pathways involving the HSA21 genes function and to discover which of them are perturbed in trisomy 21 and are relevant to neurological disorders, cognition, learning and memory in DS.

Importantly, the first altered genetic pathway involved in some DS phenotypes has been identified (Arron et al., 2006) in which two HSA21 dosage-sensitive genes are involved, DYRK1A and RCAN1/DSCR1, both located in the critical DSCR region, and impact nuclear factor of activated T cells (NFATc) activity. Transgenic mice harbouring different mutations in the NFATc transcription factors exhibit phenotypes similar to features seen in DS (Arron et al., 2006). Moreover, it has been demonstrated that DYRK1A and DSCR1 regulate NFATc and their overexpression dysregulates the NFATc pathways (Arron et al., 2006). The NFATc pathways play critical roles in vertebrate development and organogenesis of several organs (Crabtree & Olson, 2002; Graef, Che, & Crabtree, 2001), in particular the central nervous system. The signalling pathways (Fig. 3) are activated by the entry of calcium into the cell and results in activation of calcineurin, the catalytic subunit of the Ca^{2+}/calmodulin-dependent protein phosphatase PP2B. In the cytoplasm, activated calcineurin removes phosphate groups from NFATc factors. Dephosphorylated NFATc proteins enter the

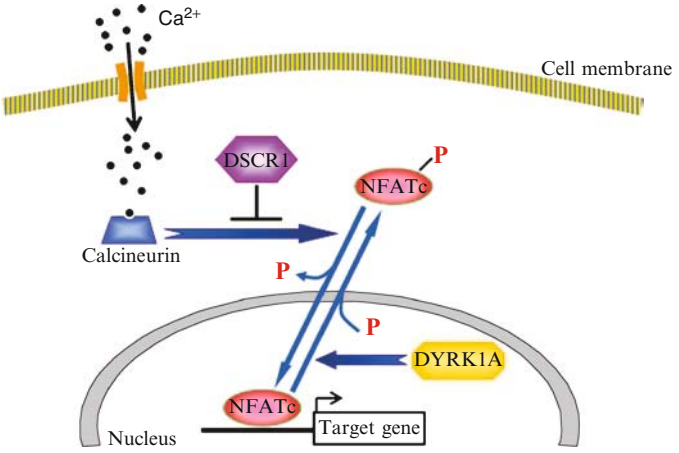

Fig. 3 Cooperation of gene-dosage imbalance of DYRK1A and DSCR1 dysregulate NFATc signalling pathway. The phosphatase calcineurin is activated by the calcium and removes phosphate groups (P) from NFATc transcription factors in the cytoplasm that allows the dephosphorylated NFATc proteins to enter into the nucleus and activate their target genes. DSCR1 is a cytoplasmic inhibitor of calcineurin and, thus, decreases NFATc dephosphorylation. In the nucleus, NFATc may be phospharylated by DYRK1A kinase and the phosphorylated NFATc returns into the cytoplasm, decreasing gene transcription activity. In DS, overexpression of DSCR1 leads to a decrease in NFATc dephosphorylation and, consequently, to a reduction in nuclear NFATc and target gene transcription. Similarly, in the nucleus, overexpression of DYRK1A leads to increase of the phosphorylated NFATc proteins and their cytoplasmic translocation, and thus to additional decrease of target gene transcription. Thus, DYRK1A and DSCR1 proteins regulate the levels of NFATc phosphorylation and, in DS, their overexpression determines the dysregulation of NFATc-dependent gene expression and their associated phenotypic features

nucleus and activate their target genes. DSCR1 encodes an inhibitor of calcineurin (Fuentes et al., 2000; Rothermel et al., 2000) and decreases NFATc dephosphorylation in the cytoplasm. Once in the nucleus, NFATc may be phosphorylated by DYRK1A serine-threonine protein kinase, and the phosphorylated forms of NFATc return into the cytoplasm, decreasing gene transcription activity.

In normal conditions, DYRK1A and DSCR1 act synergistically to control phosphorylation levels of the NFATc and NFATc-regulated gene transcription (Fig. 3). In transgenic mice overexpressing Dyrk1A or DSCR1 alone, or both Dyrk1A and DSCR1, NFATc is mostly phosphorylated and found in the cytoplasm, suggesting that overexpression of Dyrk1A and DSCR1 reduces NFATc transcriptional activity by increase of the phosphorylated forms of NFATc proteins, which leads to their cytoplasmic localisation. Interestingly, the mice lacking NFATc2 and NFATc4, such as transgenic mice overexpressing DYRK1A and/or DSCR1, have similar phenotypes to those seen in trisomic mouse models, Ts65Dn and Ts1Cje, and DS patients, including neuronal and behavioural phenotypes (Arron et al., 2006). In agreement with this molecular pathway, significant reduced calcineurin activity is detected in DS foetal brain tissue as well as in *Drosophila* mutants that overexpress DSCR1 (Chang et al., 2003).

As described above, both DYRK1A and DSCR1 are involved in synaptic development, maturation and plasticity. Moreover, DYRKIA also appears involved in splicing control. In the nuclei, DYRK1A is localised to the nuclear speckles that represent the splicing compartment (Alvarez, Estivill, & de la Luna, 2003), and several splicing factors and proteins involved in splicing regulation are DYRK1A substrates (de Graaf et al., 2004). The splicing is a fundamental step of mRNA maturation and correct protein production, and almost all genes show alternative splicing forms. Overexpression of DYRK1A in DS may dysregulate the splicing control and may determine several developmental and functional alterations, particularly in the brain.

Interestingly, several DYRK1A substrates, including the transcription factors GLI1, ARIP4, GR, FKHR and CREB (Mao et al., 2002; Sitz, Tigges, Baumgartel, Khaspekov, Lutz, 2004; Woods et al., 2001; Yang et al., 2001), are involved in the MAPK pathway. In addition, DSCR1 inhibites calcineurin and, indirectly, affects its substrates, which include dynamin and SYNJ1 (Cousin, Tan, & Robinson, 2001), and is a critical point of connection between the calcineurin and the MAPK pathways (Rothermel et al., 2003). Other genes of the chromosome 21 are involved in the MAPK pathway, such as SUMO3, involved in sumoylation, a post-translational process regulating protein function and activity level, and NRIP1 (also known as RIP140), a steroid hormone co-repressor. The MAPK pathway is involved in regulation of synaptic plasticity and memory (Sweatt, 2001; Sweatt & Weeber, 2003; Thomas & Huganir, 2004). Dysregulation of MAPK affects LTP, spatial learning, and context discrimination, which correspond to some defects observed in Ts65Dn mice (Hyde, Frisone, & Crnic, 2001; Kleschevnikov et al., 2004; Stasko & Costa, 2004) and in children with DS (Pennington, Moon, Edgin, Stedron, & Nadel, 2003).

Dysregulation of calcineurin activity is associated with cognitive and behavioural deficits, including spatial learning and context discrimination (Lee & Ahnn, 2004;

Mansuy, 2003) that are related to DS. Calcineurin interacts with dynamin in a Ca2+-dependent fashion during depolarisation-induced vesicle recycling (Liu, Sim, & Robinson, 1994) and, in *Drosophila*, disruption of this interaction blocks endocytosis and impairs neurotransmission (Kuromi, Yoshihara, & Kidokoro, 1997). Inhibition of calcineurin reduces the level of synaptic vesicle recycling as well as the total vesicle pool size in synaptic terminals (Kumashiro et al., 2005), indicating a potential role for endogenous calcineurin inhibitors in regulating synaptic transmission. It is plausible, given its role in the regulation of calcineurin activity, that overexpression of DSCR1, as observed in DS and AD, may adversely affect at least two calcineurin-dependent pathways by blocking calcineurin activity. Firstly, elevated levels of DSCR1 may disrupt endocytosis and vesicle recycling due to the inhibition of calcineurin-dependent dephosphin dephosphorylation, and secondly, may contribute to the hyperphosphorylation of Tau by reducing calcineurin phosphatase activity. Indeed, while short-term induction of the DSCR1 protein can provide stress protection in neurons, it has been proposed that long-term induction causes gradual accumulation of hyperphosphorylated tau protein, leading to AD (Ermak et al., 2001). DSCR1 knock-out mice showed increased enzymatic activity of both calcineurin and protein phosphatase 1 (PP1) and decreased phosphorylation of the calcineurin substrate DARPP-32, consistent with an elevation in calcineurin activity in the hippocampus of DSCR1 knock-out mice (Hoeffer et al., 2007), demonstrating a critical role for DSCR1 in the proper manifestation of memory and in MR in DS.

9 Potential Directions for Mental Retardation Therapeutics in Down Syndrome

The functional genomic advances for generating gene–phenotype correlations are of the most interest towards the identification of potential targets in the molecular pathways involved in DS phenotype. The efforts are focused particularly on the pathways involved in learning and memory processes in mouse models of DS towards identification of targets for therapeutics that will correct the MR features in DS patients.

It is known that individuals with DS present neuropathology indistinguishable from those with AD (Mann & Eisiri, 1989), including loss of acetylcholine and related enzymes in the hippocampus and throughout the neocortex. The cholinergic degeneration found in AD and DS led to the suggestion that pharmacological treatments directed at cholinergic systems might attenuate the degree of dementia in AD (Smith & Swash, 1978). Today, the most widely used treatment for dementia in AD is the administration of acetylcholinesterase inhibitors (AChEI), which enhance cholinergic transmission. Donepezil is a selective AChEI, which produces clinical improvement in patients with dementia in AD (Kaduszkiewicz, Zimmermann, Beck-Bornholdt, & van den Bussche, 2005). Donepezil administration improved cognition in several animal models of impaired learning including aged rodents, in animals with experimentally induced cholinergic deficits and in mouse models of AD (Yoo, Valdovinos, & Williams, 2007). Several studies (Prasher, 2004) have

addressed the treatment of cognitive decline related to dementia in DS. These studies have reported limited improvements after donepezil treatment in global functioning, cognitive skills and adaptive behaviour in people with DS. Donepezil administration also produces some improvement in language skills in adults (Heller et al., 2003) and children (Heller et al., 2004) with DS.

Therapeutic interventions have been tried out to improve learning in Ts65Dn mice. Oestrogen administration improved learning performance in the T-maze and reversed cholinergic impairment in 11–15-month Ts65Dn females (Granholm et al., 2002). Several studies have also suggested that the deficits in learning and memory seen in the Ts65Dn mouse might be partially due to increased inhibition at the synaptic level. Ts65Dn mice show a decrease of excitatory synapses (Kurt et al., 2000, 2004) and in synapse connectivity (Belichenko et al., 2004; Hanson et al., 2007). Furthermore, two studies provided evidence of increases in $GABA_A$-receptor-mediated inhibition in Ts65Dn mice. In Ts65Dn mice, it has been shown that evoked LTP in granule cells of the dentate gyrus is reduced due to an increased GABA-dependent inhibition of these neurons (Kleschevnikov et al., 2004). Moreover, it has been found that there is a significant reduction of the amount of theta-burst stimulation (TBS)-induced LTP in Ts65Dn mice that could be rescued via picrotoxin application. Therefore, an increase in $GABA_A$-mediated inhibition or in plasticity of the inhibitory circuitry in Ts65Dn mice may underlie the cognitive deficits found in these mice (Costa & Grybko, 2005).

Recently, it has been demonstrated that administering the $GABA_A$ antagonists, picrotoxin, bilobalide or pentylenetetrazol (PTZ), restored cognition and LTP in the Ts65Dn mouse, and suggested that this positive effect could me mediated by reducing inhibition in this mouse (Fernandez et al., 2007). The chronic systemic administration of noncompetitive $GABA_A$ antagonists leads to a persistent, post-drug recovery of cognition in Ts65Dn mice, as well as recovery of deficits in LTP. Only 10 days of drug treatment resulted in improved performance in the novel object recognition and spontaneous alternation in the T-maze tasks, an improvement that persisted for several months after drug treatment ended (Fernandez et al., 2007). More recently, these studies were confirmed using the non-competitive $GABA_A$ antagonist PTZ which rescued Ts65Dn performance in the Morris water maze (Rueda, Florez, & Martinez-Cué, 2008).

These findings suggest that excessive GABAergic inhibition of specific brain circuits is a potential cause of MR in DS, and that $GABA_A$ antagonists may be useful therapeutic tools to facilitate functional changes that can ameliorate cognitive impairment in children and young adults with the disorder.

More recently, it has been demonstrated that acute injections of the NMDA receptor antagonist memantine rescue performance deficits in the Ts65Dn mouse model of DS on a conditioning fear test (Costa, Scott-McKean, & Stasko, 2008). One target of memantine is the NMDA receptor, whose function is predicted to be perturbed by the integrated effects of increased expression of several HSA21 genes, including RCAN1, APP, ITSN1 and DYRK1A, all of which are being intensively studied for relevance to DS.

It is known that NMDA receptors are among the targets of calcineurin. It has been demonstrated that the pharmacological inhibition of calcineurin activity leads

to increased NMDA receptors mean open time and opening probability (Lieberman & Mody, 1994). Theoretically, such modulation of kinetic parameters should lead to an increase in inhibition of NMDA receptors-mediated currents by open channel blockers, including the noncompetitive NMDA receptors antagonist MK-801. Accordingly, conditional calcineurin null-mutant mice display increased responses to the locomotor-stimulating effects of MK-801 (Miyakawa et al., 2003).

DSCR1 knock-out mice have pronounced spatial learning and memory deficits (Hoeffer et al., 2007). These deficits were similar to those found in mice with inducible, hippocampal-restricted overexpression of constitutively active calcineurin (Mansuy, Mayford et al., 1998; Mansuy, Winder et al., 1998) and the direct opposite of learning behaviours of animals in which calcineurin was inhibited by either transgenic expression of a calcineurin inhibitory domain or application of antisense oligonucleotides (Ikegami & Inokuchi, 2000; Malleret et al., 2001). This suggests that DSCR1 provides a constraint on calcineurin activity during learning and memory and that this constraint is absent in the DSCR1 knock-out mice. Moreover, recent findings indicate that acute blockade of calcineurin activity improves memory and cognitive function in AD model mice (Dineley, Hogan, Zhang, & Taglialatela, 2007). These findings strongly suggest that DSCR1 facilitates synaptic plasticity and memory by constraining phosphatase signalling via inhibition of calcineurin and its downstream target PP1. Thus, DSCR1 represents an important potential therapeutic target for the treatment of numerous neurological disorders whose pathologies involve the dysregulation of calcineurin (Hoeffer et al., 2007).

In addition, efforts could also be concentrated on the microRNAs of the HSA21 to elucidate their biological role in DS, because it has been demonstrated recently that microRNAs show an increasing importance in the gene expression control and seem to play a fundamental role in diverse biological and pathological processes, including cell proliferation, differentiation, apoptosis, carcinogenesis, and cardiovascular disease (Bushati & Cohen, 2007; Wang et al., 2007). Considering the hypothesis that trisomic 21 gene-dosage overexpression of HSA21-derived miRNAs results in the decreased expression of specific target proteins and contributes, in part, to features of the neuronal and cardiac DS phenotype, HSA21-derived miRNAs may provide novel therapeutic targets in the treatment of individuals with DS (Kuhn et al., 2008).

This indicates that studies of variation of gene expression and its genomic regulation, and functional studies of these genes and other conserved DNA elements, are two fundamental research priorities that may also provide potential future directions for MR therapeutics in DS.

10 Conclusion and Perspectives

Partial trisomies of HSA21 or MMU16 allowed genetic dissection of DS phenotypes, particularly the neurological ones, the goal of which is the identification of candidate genes contributing to neurological and behavioural phenotypes, and to MR in DS.

The gene expression studies in mouse models and human have shown similar genotype/phenotype correlations. The highly parallel outcomes that result when the same evolutionarily conserved genetic programmes are perturbed in mice and human validate the recent studies that focus on DS phenotypes, particularly neurological alterations. Thus, these studies indicate that brain dysfunctions and MR may be due to over-dosage of genes involved, directly or indirectly, in brain developmental processes throughout neurogenesis, neuronal growth and neuronal differentiation.

All these studies identified some genes which are overexpressed in the brain and involved in brain development, learning and memory as candidate genes for MR in DS. The increased information about function of the proteins encoded by these genes, their interaction with other proteins and their involvement in regulatory and metabolic pathways is giving a clearer view of the origin of the MR in DS. This leads to the identification of potential targets in the molecular altered pathways involved in MR pathogenesis that may be potentially corrected, in the perspective of new therapeutic approaches.

Furthermore, the regulation of gene expression by microRNAs or small interfering RNAs provide exciting possibilities for exogenous correction of the aberrant gene expression in DS and also provide potential directions for clinical therapeutics of MR.

Given the new pharmacotherapies for cognitive impairment in a mouse model of DS and the relevance of these findings to the treatment of cognitive deficits in the human DS population, substantial interest emerges in clinical settings and trials in DS and has created substantial interest of the scientific and medical community towards a novel biomedical era for therapeutics of MR in DS.

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