

## Trisomy 21 and Down syndrome - A short review

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Received June 29, 2007 – Accepted August 20, 2007 – Distributed May 31, 2008

### Abstract

Even though the molecular mechanisms underlying the Down syndrome (DS) phenotypes remain obscure, the characterization of the genes and conserved non-genic sequences of HSA21 together with large-scale gene expression studies in DS tissues are enhancing our understanding of this complex disorder. Also, mouse models of DS provide invaluable tools to correlate genes or chromosome segments to specific phenotypes. Here we discuss the possible contribution of HSA21 genes to DS and data from global gene expression studies of trisomic samples.

*Keywords:* Down syndrome, trisomy, HSA21, gene-expression analysis.

### Trissomia do 21 e Síndrome de Down: uma breve revisão

#### Resumo

Embora os mecanismos moleculares que causam a síndrome de Down (SD) não sejam totalmente conhecidos, a caracterização de genes e seqüências não gênicas conservadas do HSA21 e os estudos de expressão em grande escala em amostras de pacientes com SD estão aumentando o entendimento da síndrome. Por outro lado, os modelos murinos da SD provêm ferramentas valiosas para correlacionar genes ou segmentos cromossômicos a características fenotípicas específicas. Nesta revisão, são discutidas as possíveis contribuições dos genes do HSA21 à SD e os dados de estudos de expressão gênica global de amostras trissômicas.

*Palavras-chave:* Síndrome de Down, trissomia do 21, HSA21, análise da expressão gênica.

#### 1. Introduction

Trisomy 21 is the most common genetic cause of mental retardation and one of the few aneuploidies compatible with post-natal survival. It occurs in 1 out of 700 live births in all ethnic groups (Epstein, 2001). The vast majority of meiotic errors leading to the trisomic condition occur in the egg, as nearly 90% of cases involve an additional maternal chromosome (Hassold and Sherman, 2000). Besides mental retardation, present in every individual with Down syndrome (DS), trisomy 21 is associated with more than 80 clinical traits including congenital heart disease, duodenal stenosis or atresia, imperforate anus, Hirschprung disease, muscle hypotonia, immune system deficiencies, increased risk of childhood leukemia and early onset Alzheimer's disease (Epstein et al., 1991). The severity of each of the phenotypic features is highly variable among the patients. In this sense, the identification of single nucleotide polymorphisms (SNPs) on HSA21 provides a tool to study the contribution of the allelic variability to the phenotypic variability (Deutsch et al., 2001).

It is widely assumed that the DS complex phenotype results from the dosage imbalance of the genes located on HSA21. The products of these genes act directly or indirectly, by affecting the expression of disomic genes.

This hypothetical model requires different experimental approaches that include, but are not restricted to, the complete characterization of HSA21 genes and non-coding sequences and the analysis of the global gene expression changes induced by trisomy in every tissue/cell type available and at different developmental stages.

#### 2. Human Chromosome 21

The genetic nature of DS together with the relatively small size of HSA21 encouraged scientists to concentrate efforts towards the complete characterization of this chromosome in the past few years. The almost complete DNA sequence of the long arm (21q) of HSA21 was determined and published in *Nature* (Hattori et al., 2000). This represented a breakthrough for research in DS, greatly assisting in the identification of every gene and non-coding sequence of 21q.

The length of 21q is 33.5 Mb and approximately 3% of its sequence encodes for proteins. The initial analysis of 21q revealed 225 genes (127 known genes and 98 putative novel genes predicted in silico) and 59 pseudogenes (Hattori et al., 2000). Although the precise gene catalogue has not yet been conclusively determined, Gardiner et al.

(2003) have estimated 364 genes and putative genes from the finished sequence of HSA21. The proteins encoded by these genes fall into several functional categories including transcription factors, regulators and modulators (18 genes); proteases and protease inhibitors (6 genes); ubiquitin pathway (4 genes); interferons and immune response (9 genes); kinases (8 genes); RNA processing (5 genes); adhesion molecules (4 genes); channels (7 genes); receptors (5 genes); and energy metabolism (4 genes). Interestingly, ~1% of the HSA21 corresponds to conserved non-genic (CNG) sequences, that is, sequences that are not “functionally” transcribed and do not correspond to protein-coding genes (Dermitzakis et al., 2002; Dermitzakis et al., 2004). The significant conservation of these sequences indicates that they are functional, although their function is unknown.

The identification and characterization of HSA21 genes may improve our understanding of the molecular basis of the disease. Even before the complete sequence of 21q was determined, an intensive work started towards the characterization of HSA21 genes. The existence of a “Down Syndrome Critical Region” (DSCR), a small segment of HSA21 that contains genes responsible for many features of DS, has dominated the field of DS research for three decades. Accordingly, a number of genes contained in this ~5.4 Mb region have been extensively studied as an attempt to find out their potential contributions to DS. Two of these genes are DSCR1 and DSCR2.

The DSCR1 (“Down Syndrome Critical Region 1”) protein, now renamed RCAN1 (from “Regulator of Calcineurin 1”) (Davies et al., 2007) is over-expressed in the brain of Down syndrome fetuses and interacts physically and functionally with calcineurin A, the catalytic subunit of the Ca(2+)/calmodulin-dependent protein phosphatase PP2B (Fuentes et al., 2000; Harris et al., 2005). RCAN1 is highly expressed in the human brain and heart suggesting that its overexpression may be involved in the pathogenesis of Down syndrome, particularly mental retardation and/or cardiac defects (Fuentes et al., 1995). Previous studies identified conserved residues involved in the subcellular location of RCAN1 (Pfister et al., 2002) and provided evidence that it may play a functional role in the nucleus, probably as a regulator of transcription (Silveira et al., 2004). Recently, Arron et al. (2006) reported that the genes RCAN1 and DYRK1A, both contained within the DSCR, act synergistically to prevent the nuclear occupancy of NFATc transcription factors. They suggested that the 1.5-fold increase in dosage of RCAN1 and DYRK1A cooperatively destabilizes a regulatory circuit, leading to reduced NFATc activity and many of the features of Down syndrome.

The gene DSCR2 (“Down Syndrome Critical Region 2”) is highly expressed in all proliferating tissues and cells, such as fetal tissues, adult testis and cancer cell lines (Vidal-Taboada et al., 2000). The intracellular localization and proteolytic cleavage of the protein have been carefully studied (Abrão-Possik et al., 2004; Vesa et al., 2005). Hirano et al. (2005) have recently designat-

ed DSCR2 as “Proteasome Assembling Chaperone-1” (PAC1). PAC1 and PAC2 are chaperones that function as heterodimers in the maturation of mammalian 20S proteasomes. Overexpression of PAC1 or PAC2 accelerates the formation of precursor proteasomes, whereas knockdown by short interfering RNA impairs it, resulting in poor maturation of 20S proteasomes (Hirano et al., 2005). Thus, the product of the gene DSCR2 is involved in the correct assembly of 20S proteasomes.

Of note, there are eighteen genes located on HSA21 that encode transcription factors and co-regulators/modulators of transcription. These proteins are directly and indirectly involved in transcription regulation and alterations in their expression levels could impact the expression of downstream targets. This notion is supported by a number of studies reporting the dysregulation of disomic genes in DS tissues (see references below). The identification of the targets of these regulators is of prime importance to assess their contribution to the molecular pathogenesis of DS.

Despite the great efforts made in the search for a “critical region”, the existence of individual loci on HSA21 responsible for producing the clinical features of DS has not been demonstrated (Shapiro, 1999). Indeed, a recent study provided the evidence that trisomy for the DSCR is necessary but not sufficient for the brain phenotypes observed in trisomic mice (Olson et al., 2007). Thus, although HSA21 genes are likely to contribute to DS, the abnormalities seen in the patients are multifactorial conditions (Shapiro, 1999) and are the result of genetic, environmental and stochastic influences (Reeves et al., 2001). Besides the complete characterization of HSA21 genes, we need to understand the transcriptional effects caused by trisomy 21.

### 3. Transcriptional Consequences of Trisomy 21

A model for the transcriptional consequences of trisomy has been proposed recently (FitzPatrick, 2005). An extra copy of HSA21 genes would result in a 1.5-fold increase in the expression of many of them, some of which will produce a phenotypic effect directly. Overexpression of HSA21 genes that encode *trans*-acting factors is expected to induce a mis-regulation of disomic genes. The primary gene-dosage effects as well as the *trans*-acting gene-dosage effects will produce a phenotypic effect, which will result in a tertiary apparent “mis-regulation” of disomic genes. The presence of CNG sequences on HSA21 indicates that they may also have a role in the generation of DS phenotypes although this has yet to be confirmed. Some of the genes for which evidence indicates over-expression in DS brain are listed in Table 1.

Several studies have reported a generalized overexpression of triplicated genes at the mRNA level in mouse models of DS (Amano et al., 2004; Lyle et al., 2004; Kahlem et al., 2004; Dauphinot et al., 2005). Interestingly, studies performed on human trisomic tissues indicate that only a subset of HSA21 genes is over-expressed relative to euploid controls and that the

**Table 1.** HSA21 genes overexpressed in DS brain.

Gene	Functional categories*	Reference
DSCAM	Adhesion molecules	Saito et al., 2000
HMG14	Chromatin structure	Epstein, 2001
DSCR1	Miscellaneous	Fuentes et al., 2000
PKNOX1	Transcription factors	Sanchez-Font et al., 2003
APP	Miscellaneous	Epstein, 2001
BACH1	Transcription factors	Ferrando-Miguel et al., 2003a
DYRK1A	Kinases	Dowjat et al., 2007
S100 $\beta$	Miscellaneous	Epstein, 2001
ERG	Transcription factors	Shim et al., 2003
ETS2	Transcription factors	Wolvetang et al., 2003
SOD-1	Oxygen metabolism	Gulesserian et al., 2001
IFNAR2	Receptors	Ferrando-Miguel et al., 2003b
BACE-2	Proteases	Barbiero et al., 2003
SYNJ1	Phosphatases	Arai et al., 2002

\*functional categories were assigned as described previously (Gardiner and Davisson, 2000).

increase in expression may be different from the expected ~1.5-fold (FitzPatrick et al., 2002; Tang et al., 2004; Mao et al., 2005). Also, the set of over-expressed HSA21 genes differs across the trisomic cell types (Li et al., 2006). These findings indicate that the presence of three copies of a gene does not necessarily result in over-expression and that other factors (e.g. developmental stage, tissue-specific differences) also affect gene expression.

The extensive variation in the expression of HSA21 genes observed among unaffected individuals (Deutsch et al., 2005) might underlie some of the phenotypic variability seen in the patients. The determination of which genes are significantly over-expressed in DS is largely dependent on the degree of gene-expression variation: while some HSA21 genes show little or no overlap in the distribution of expression values between DS and control samples, others show overlapping distributions with varying degrees (Prandini et al., 2007). Furthermore, a recent report indicates that many HSA21 genes are likely to be compensated in DS and some of them are highly variable among individuals (Ait Yahya-Graison et al., 2007). The genes with minimal expression overlap are over-expressed in DS and probably associated with the constant DS features; those with partially overlapping expression distributions could account for the variable features. Assessment of this natural gene-expression variation in several DS tissues will provide information to identify candidate genes. In addition, the characterization of the protein profiles of trisomic samples will be of importance to see how well the transcript levels correlate with the corresponding protein products.

The increase in expression of some HSA21 genes would induce changes in the global gene expression pattern that ultimately contribute to the DS phenotypic features. A number of studies have reported dysregulation of disomic genes in DS tissues (FitzPatrick et al., 2002; Tang et al., 2004; Mao et al., 2005). Different sets

of non-HSA21 genes show up- or down regulation as a consequence of chromosomal imbalance. It is likely that some (if not all) the DS phenotypic features are not directly attributable to single gene(s) but are at least in part the result of a more generalized gene dysregulation caused by the triplicated chromosome. A recent study in fetal hearts of trisomic subjects provided additional evidence supporting the existence of a dysregulation of non-HSA21 genes associated with the primary gene-dosage effect. Interestingly, functional clustering of dysregulated genes revealed down-regulation of genes encoding mitochondrial enzymes and up-regulation of genes encoding extracellular matrix proteins in DS, suggesting an association of these alterations with the heart defects (Conti et al., 2007). As each tissue is characterized by a distinct proteome, we expect that different sets of disomic genes will be subject to dysregulation in the various tissues. Therefore, every tissue/cell type available should be investigated.

We have analyzed the gene expression profile of DS lymphocytes using SAGE "Serial Analysis of Gene Expression". SAGE is a powerful technique that allows the characterization of global gene expression profiles (Velculescu et al., 1995). In the SAGE method, 10-base tags are obtained from each transcript, concatenated, and sequenced. By cataloging tags along with their frequencies and identifying corresponding genes, we can estimate the expression level of thousands of genes simultaneously. Among the significantly differentially expressed SAGE tags, many corresponded to genes involved in transcription, RNA processing, signaling, immune response and lipid metabolism. Our results suggest that trisomy 21 induces a modest dysregulation of disomic genes that may be related to the immunological perturbations seen in DS (Sommer et al., 2008). In a previous study, we used SAGE to generate a comprehensive expression profile of DS leukocytes (Malago-Junior et al., 2005). The avail-

ability of the SAGE data may aid in the identification of gene signatures associated with specific treatments and therapeutic interventions of DS blood cells.

#### 4. Mouse Models of DS

The studies performed on human trisomic tissues are restricted because of practical and ethical reasons. In contrast, mouse models of human disorders provide access to all tissues at all stages of development. Regardless of the species-specific differences between human and mouse, they have become indispensable tools for dissecting the phenotypic consequences of imbalances that affect single genes or chromosome segments. Although the current murine models of DS do not show all the features of the syndrome, they have greatly enhanced our understanding of the cellular and biochemical mechanisms involved.

Mouse orthologues of chromosome 21 genes are located on three chromosomes: MMU16 (~23 Mb), MMU17 (~1.1 Mb), and MMU10 (~2.3 Mb). The most widely used models are the segmental trisomy strains Ts65Dn and Ts1Cje that contain several HSA21 orthologues in three copies. Both display overlapping phenotypes that parallel those seen in DS, including learning and behavioral deficits (Reeves et al., 1995; Sago et al., 1998). Two additional mouse models have been developed recently. O'Doherty et al. (2005) created the "transchromosomal" mouse Tc1, which carries an almost complete copy of HSA21 and have heart defects like those seen in DS patients, together with spatial learning and memory deficits. The segmental trisomy mouse model Ts1Rh is trisomic for the DSCR (Olson et al., 2004). Other mouse models trisomic for smaller HSA21 syntenic regions or even single genes should be generated to assess their putative contribution to the DS specific abnormalities.

#### 5. Conclusions and Perspectives

The molecular mechanisms leading to DS are incompletely understood. The inconsistencies found in large scale transcriptome studies of trisomic tissues along with the extensive gene-expression variation of HSA21 genes indicate that more research is needed before we can elucidate the numerous pathogenic mechanisms associated with this complex disorder. In this sense, mouse models of DS provide invaluable tools to correlate genes or chromosome segments to specific phenotypes. It will be some time before we can start considering the development of strategies for prevention and treatment of some DS related pathologies.

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