Journal of Neurochemistry, 2006, **97** (Suppl. 1), 104–109 doi:10.1111/j.1471-4159.2005.03624.x

SPECIAL ISSUE

Transcriptional disruptions in Down syndrome: a case study in the Ts1Cje mouse cerebellum during post-natal development

M.-C. Potier,* I. Rivals,† G. Mercier,* L. Ettwiller,* R. X. Moldrich,* J. Laffaire,* L. Personnaz,† J. Rossier* and L. Dauphinot*

**Unité Mixte de Recherche, 7637 Centre National de la Recherche Scientifique, Neurobiology and*
†Equipe de Statistique Appliquée, Ecole Supérieure de Physique et de Chimie Industrielles, Paris, France

Abstract

To understand the aetiology and the phenotypic severity of Down syndrome, we searched for transcriptional signatures in a substructure of the brain (cerebellum) during post-natal development in a segmental trisomy 16 model, the Ts1Cje mouse. The goal of this study was to investigate the effects of trisomy on changes in gene expression across development time. The primary gene-dosage effect on triplicated genes (~1.5) was observed at birth [post-natal day 0 (P0)], at P15 and P30. About 5% of the non-triplicated genes were significantly differentially expressed between trisomic and control cerebellum, while 25% of the transcriptome was modified during post-natal development of the cerebellum. Indeed, only 165, 171 and 115 genes were dysregulated in trisomic cerebellum at P0, P15 and P30, respectively. Surprisingly, there were only three genes dysregulated in development and in trisomic animals in a similar or opposite direction. These three genes (*Dscr1, Son* and *Hmg14*) were, quite unexpectedly, triplicated in the Ts1Cje model and should be candidate genes for understanding the aetiology of the phenotype observed in the cerebellum.

Keywords: cerebellum, development, Down syndrome, microarray, transcriptome.

J. Neurochem. (2006) **97** (Suppl. 1), 104–109.

Down syndrome, the most frequent genetic cause of mental retardation occurring in ~1 in 800 newborns, results from the presence of three copies of chromosome 21 (Trisomy 21) (Lejeune *et al*. 1959). This dosage imbalance of around 300 genes causes dysfunction of developmental and physiological processes, leading to a complex phenotype defined by several clinical features which are variable in their number and intensity (Epstein *et al.* 1991). Because mental retardation is present in all patients, the brain has been the subject of particular interest.

A key question for understanding the aetiology of Down syndrome is whether the range of symptoms results from a developmental instability as a result of the cumulative effects of a large number of genes (major change in gene expression) or from a dosage effect on a small number of triplicated genes that negatively impact the development and working of the brain. If the effect is restricted to a small number of genes, then the other major goal is to demonstrate if Down syndrome is the consequence of a snowballing effect during development on multiple independent processes, or rather a cascading effect, starting with an initial insult propagating through gene expression network during development (Fig. 1). The 300 genes that are at dosage imbalance (three copies) are not all expressed across development time but, when present, they will be over expressed as compared with normal tissue. For example, gene 1 is present and over expressed all over development, while genes 2 and 3 are only present and over expressed during a short time window. If these three genes are involved in three different processes, then, during development, more and more processes will be altered (snowballing effect). Alternatively, if gene 2, that is present and over expressed in a short time window, regulates

Received August 1, 2005; revised manuscript received September 28, 2005; accepted November 2, 2005.

Address correspondence and reprint requests to Marie-Claude Potier, Unité Mixte de Recherche 7637, Centre National de la Recherche Scientifique, 10 rue Vauquelin, 75005 Paris, France.
E-mail: marie-claude.potier@espci.fr

Abbreviations used: INV, inverse; P0, post-natal day 0; QPCR, quantitative PCR; SIM, similar.

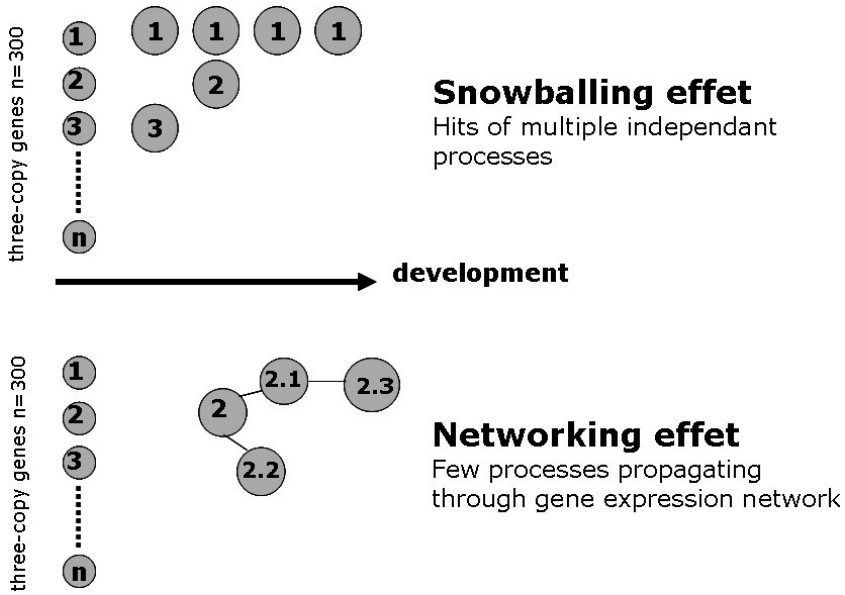

Fig. 1 Proposed model for the transcriptional disruptions in Down syndrome: combination of a snowballing effect and a networking effect. Circles represent respective gene expression.

two-copy genes 2.1, 2.2 and 2.3, then the effect of gene 2 will propagate through a gene expression network during development (networking effect). Hence, the initial insult, which is the over expression of three-copy genes, might be unrelated to the phenotype which could result from secondary effects (networking effect) in development.

For this reason, large scale gene expression studies during development in substructures of the brain are of particular interest. Several gene expression studies such as SAGE, DNA microarrays and quantitative PCR (QPCR) have been published (Chrast *et al*. 2000; FitzPatrick *et al*. 2002; Mao *et al*. 2003; Saran *et al*. 2003; Amano *et al*. 2004; Kahlem *et al*. 2004; Lyle *et al*. 2004; reviewed in FitzPatrick 2005) but none of them combined the effects of Trisomy 21 with the effects of development.

The consequences of Trisomy 21 on the transcriptome would either be a generalized modification of gene expression (developmental instability), a more restricted dysregulation involving a smaller number of genes (gene-dosage effect) or an intermediate situation. If the transcriptome modifications were to be restricted, then candidate genes (within or outside of chromosome 21) could be listed for defining new therapeutic targets. In the developing brain, candidate genes would be involved in neurogenesis, neuronal differentiation, myelination or synaptogenesis.

Systematic studies of gene expression in brain during development, particularly at post-natal stages, are only possible in animal models. Mouse models for Down syndrome are either trisomic for single genes, for several genes or for a large segment corresponding to the distal part of chromosome 16, orthologous to a large portion of human chromosome 21 (Gardiner *et al*. 2003; Antonarakis *et al*. 2004). Two models of segmental trisomy 16 have been generated, the Ts65Dn and the Ts1Cje mice (Reeves *et al*. 1995; Sago *et al*. 1998). These models have revealed previously unknown phenotypes that may be relevant to Trisomy 21, such as a substantial loss of glutamatergic granule cells in the internal layer of the adult cerebellum (Baxter *et al*. 2000; Olson *et al*. 2004).

We decided to focus on the cerebellum because a phenotype had been clearly described in adult mouse models of Down syndrome and in patients. In addition, because post-natal development of the cerebellum takes place during the first 20 days after birth, the modifications of the transcriptome in mouse models were studied during post-natal development of the cerebellum. During this period, granule cells, which represent about 40% of the total number of neurons of the mature cerebellum, proliferate, migrate and differentiate from the external to the internal layer, and Purkinje cells develop their dense dendritic trees, making connections with other cells.

Finally, if we were able to define candidate genes, post-natal treatment would be easier to test in mice and eventually could be applicable to humans.

The Ts1Cje Down syndrome model was used in the present study after backcrossing the mice on to a pure genetic background to reduce variability in gene expression. Ts1Cje mice carry a segmental duplication of the syntenic region orthologous to human chromosome 21 from *Sod1* to *Znf295*, including about 95 genes. Differential gene expression was studied at post-natal day 0 (P0), P15 and P30 in the cerebellum using Affymetrix U74Av2 microarrays. Expression of genes was also measured in parallel by QPCR.

Five groups of differentially expressed genes were identified: the first two groups were related to development while the other three groups contained genes differentially expressed between trisomic and control cerebellum at the three time points studied. In addition, by exploring the

intersections between these five groups, we were able to define subgroups of genes that are similarly or inversely dysregulated in trisomic animals and during development.

Materials and methods

Experimental procedures have been described previously (Dauphinot *et al*. 2005). Briefly, pools of RNA were prepared from three pairs of euploid and trisomic Ts1Cje male siblings from two litters at each developmental stage (P0, P15 and P30). Ts1Cje mice were bred on a C57BL/6 background (11 backcrosses). Twenty micrograms of RNA from each pool were labelled using the commercial procedure and hybridized to the U74A version 2 microarrays (Affymetrix Inc., Santa Clara, CA, USA). Raw data have been deposited on the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE1611. Filtered data (8287 expressed genes and ESTs out of around 12 000 present on the chip) were submitted to ANOVA according to Dauphinot *et al*. (2005). Briefly, we used a parameterization which models the effects of development in euploid mice (P15/P0 and P30/P0), as well as the effect of trisomy at each developmental stage (Ts1/Eu at P0, P15 and P30).

Results

Expression of triplicated genes in trisomic animals

Using QPCR, we showed previously that triplicated genes are over expressed in trisomic cerebellum as compared with control cerebellum, with a mean ratio of 1.56, 1.43 and 1.55 at P0, P15 and P30, respectively (Dauphinot *et al*. 2005). Further analysis of microarray data revealed that a small proportion of the triplicated genes showed ratios above 1.5 but never below 1, indicating that, if compensation is active in the trisomic condition, then it is very subtle at the RNA level.

Global gene expression variations between trisomic and euploid cerebellum at P0, P15 and P30

The number of genes that were significantly differentially expressed between trisomic and control animals ($\alpha = 1\%$) is 419 at P0, 339 at P15 and 257 at P30, with an enrichment in genes involved in development and cell differentiation as demonstrated in the first analysis (Dauphinot *et al*. 2005). This represents about 5% of the expressed genes present on the chip, clearly indicating that not all the transcriptome is modified in trisomic animals. More genes were dysregulated in trisomic animals at birth and at P15 than at P30, suggesting that, beyond 15 days, when development is nearly achieved, compensation mechanisms take place.

Only 32 genes were differentially expressed (Ts1/Eu) at all three developmental stages (Fig. 2d and supplemental Table S1). These genes are involved in development and cell differentiation (Dauphinot *et al*. 2005). Six of them are constantly over expressed during post-natal development. Three are triplicated genes: *Dscr1* or *calcipressin 1*, *Hmg14* or *Hmgn1* and *Son*.

Calcipressins are putative inhibitors of calcineurin, a calcium-activated serine/threonine phosphatase that regulates neuronal activity. Calcipressins modulate the pattern of

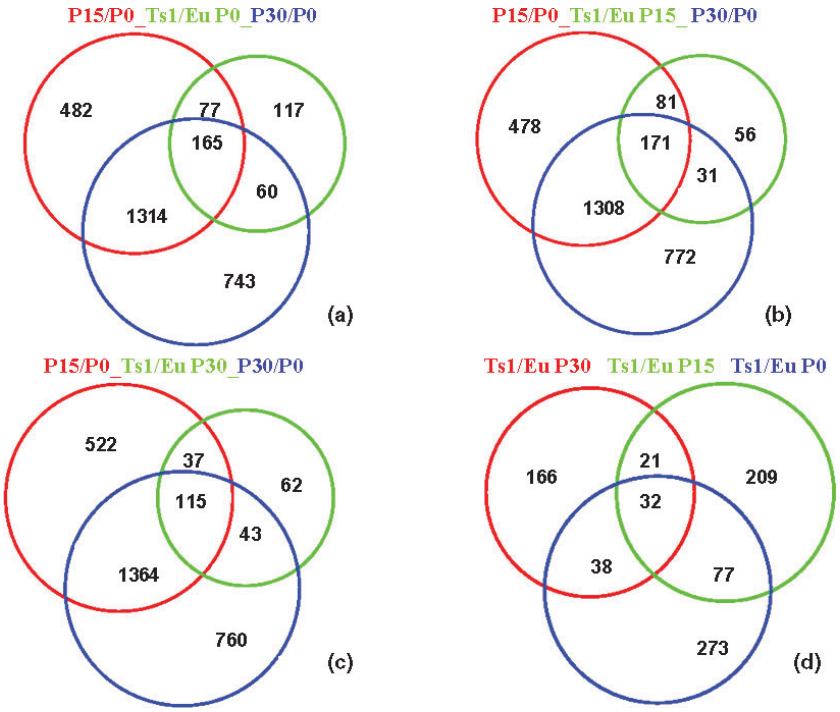

Fig. 2 Venn diagrams indicating the number of genes dysregulated during development and in trisomic animals at P0 (a), P15 (b) and P30 (c). (d) Genes dysregulated in trisomic animals at P0, P15 and P30.

calcineurin-dependent gene expression and could thus influence calcineurin activity beyond calcium (Fuentes *et al*. 1995; Ryeom *et al*. 2003). *Calcipressin 1* is expressed in the cerebellum in the adult, mostly in Purkinje cells.

Hmg14 is a member of the high-mobility group of N proteins that specifically bind to the nucleosome core particles, where it unfolds chromatin, playing a crucial role in transcription and DNA repair. It is not yet clear if HMGN proteins are activated when many genes need to be transcribed, or if each HMGN protein can modulate the expression of subsets of genes (West 2004). At the molecular level, *Hmgn1* (*Hmg14*) binds to nucleosomes and modulates the access of enzymes that phosphorylate histone H3 (Lim *et al*. 2004).

Son cell proliferation protein is an 'SR-type' protein involved in mRNA processing and gene expression. It contains a basic serine/threonine (SR) motif that is essential for splicing activity. Although *Son* targets are not yet described, some SR proteins have been involved in development and influence the selection of alternative 5′ splice sites (Wynn *et al*. 2000). *Son* is expressed in the cerebellum in adult mice, mainly in the internal granular layer, and could thus be involved in the loss of granular cells (http://chr21.molgen.mpg.de/hsa21/). More immunohistochemical data at early stages during post-natal development of the cerebellum will be necessary before drawing any conclusion.

Among the other three genes that are constantly over expressed during post-natal development is *Setdb1*, or ERG-associated protein with an SET domain (2.82, 2.88 and 1.95 at P0, P15 and P30, respectively). It is a novel histone methyltransferase that catalyzes methylation of histone H3-lysine 9 (H3-K9) during replication-coupled chromatin assembly (Sarraf and Stancheva 2004). The *Erg* gene is triplicated in the Ts1Cje mice, but was not present on the Affymetrix chip. However, using QPCR, we showed that *Erg* gene expression ratios between trisomic and control animals were over 1 at P0 and P30 (1.49 and 1.70, respectively). Thus, at P15 and P30, the two partners from the same complex followed an increase in gene expression.

Global gene expression during post-natal development of the cerebellum

Between P0 and P15, and P0 and P30, 2038 and 2282 genes, respectively, were significantly differentially expressed, representing roughly 25% of the expressed genes present on the chip. This is about five times higher than the number of genes dysregulated in trisomic animals at one particular stage of development (3–5%), suggesting that development has more impact on the transcriptome than gene-dosage imbalance.

Effect of trisomy on genes involved in post-natal development of the cerebellum

We defined five groups of genes: the first two groups contained genes differentially expressed between birth and one stage of development, namely P15/P0 (between P0 and P15) and P30/P0 (between P0 and P30). The other three groups contained genes differentially expressed between trisomic and control cerebellum at the three time points studied (Ts1/Eu at P0, Ts1/Eu at P15 and Ts1/Eu at P30). Evidently, some genes present in one group, such as P15/P0, were also present in other groups, such as Ts1/Eu at P0. Therefore, from these five groups of genes, we selected three subgroups whose profile intersected between two or more groups (Fig. 2). For example, the intersecting subgroup between P15/P0, P30/P0 and Ts1/Eu at P0 contained 165 genes (over a total of 2958), that were dysregulated in trisomic animals at P0 but also important for the post-natal development of cerebellum (Fig. 2a). The second and the third intersection subgroups (second: P15/P0, P30/P0; third: Ts1/Eu at P15 and P15/P0, P30/P0 and Ts1/Eu at P30) contained 171 and 115 genes differentially expressed in trisomic animals at P15 and P30, respectively, and were also involved in post-natal development of the cerebellum (Figs 2b and c).

Among these three subgroups, genes were further classified into two qualifiers whereby: (i) their expression profile was similar (SIM) during development and in trisomic animals by showing either a concordant increase or decrease in gene expression; (ii) their expression profile was inverse (INV) during development and in trisomic animals showing either a discordant increase or decrease in gene expression (Fig. 3 and supplementary Tables S2 and S3). The consequences for the genes belonging to one of these classes would not be the same. For the SIM class, trisomy would have a cumulative effect on development, while for the INV class trisomy would have a potentially inhibitive effect on development.

Only 12 genes were inversely regulated between P0 and P15, and P0 and P30 and few of them showed a parallel significant increase in the trisomic situation at one stage of development (supplementary Table S1).

Genes similarly or inversely regulated during development and in trisomic animals at one stage of development could be involved in the cerebellum phenotype, particularly the ones with ratios over 2 or under 0.5 (supplementary Tables S2 in yellow).

Only three genes were either similarly or inversely regulated during development and in trisomic animals at all stages (supplementary Tables S2 and S3, Fig. 3). Surprisingly, these three genes were triplicated genes from chromosome 21. The one gene similarly regulated in trisomy and during development was *Orf60* or *Son,* the cell proliferation protein that increases during development (P15/P0 = 2.19, P30/P0 = 1.55) and in trisomic animals (Ts1/Eu = 1.9 at P0, 1.44 at P15 and 1.78 at P30). The other two inversely regulated genes in trisomy and during development are *Dscr1* and *Hmg14,* with ratios of 0.64 and 0.85 between P15 and P0, respectively, and 0.6

Trend classification	Number of genes	Development trend		Trisomy trend (Ts1/Eu)		
		P15/P0	P30/P0	P0	P15	P30
Similarly augmented at P0	70					
Inversely augmented at P0	13					
Similarly reduced at P0	43					
Inversely reduced at P0	35					
Similarly augmented at P15	18					
Inversely augmented at P15	95					
Similarly reduced at P15	7					
Inversely reduced at P15	50					
Similarly augmented at P30	27					
Inversely augmented at P30	28					
Similarly reduced at P30	32					
Inversely reduced at P30	24					
Similarly augmented at P0, 15, 30	**1 (*Son*)**					
Inversely augmented at P0, 15, 30	**2 (*Dscr1, Hmg14*)**					
Similarly reduced at P0, 15, 30	0					
Inversely reduced at P0, 15, 30	0					

Fig. 3 Number of differentially expressed genes showing an increase (red) or decrease (green) in expression during post-natal development of the cerebellum between P0 and P15 (P15/P0), between P0 and P30 (P30/P0) (development trend), and between trisomic and control cerebellum at P0, P15 and P30 (Ts1/Eu_P0, Ts1/Eu_P15 and Ts1/Eu_P30, respectively) (trisomic trend). By comparing developmental and trisomic trends, the number of inversely or similarly regulated genes can be classified (e.g. similarly augmented at P0).

Table 1 Gene expression modifications of *Son, Dscr1* and *Hmg14* at P0, P15 and P30 deduced from microarray experiments and QPCR (Dauphinot *et al.* 2005)

	Ts1Cje/euploid P0		Ts1Cje/euploid P15		Ts1Cje/euploid P30	
	Microarray	QPCR	Microarray	QPCR	Microarray	QPCR
Son	1.9	1.58	1.44	1.44	1.78	1.82
Dscr1	1.32	1.62	1.58	1.49	1.48	1.48
Hmg14	1.58	1.84	1.35	1.19	1.29	1.33

between P30 and P0 for both genes. In trisomic animals, *Dscr1* and *Hmg14* showed Ts1/Eu expression ratios of 1.32 and 1.58 at P0, 1.58 and 1.35 at P15, and 1.48 and 1.29 at P30, respectively. Table 1 recapitulates the over expression ratios for the three candidate genes, obtained through microarray experiments or by QPCR (Dauphinot *et al.* 2005).

Discussion

Gene profiling combining the effects of trisomy with those of development proved to be useful for shortening the list of candidate genes involved in the cerebellar phenotype of Ts1Cje mice. The role of the three triplicated genes *calcipressin 1*, *Hmg14* and *Son* in post-natal development of the cerebellum is not yet known. Among classes of genes similarly or inversely dysregulated during development and in trisomy, is a small set with ratios that are above 2 or below 0.5 that could also be targets for future functional studies. The list of these genes is presented in yellow in supplementary Tables S2 and S3.

Previous studies on whole brain of Ts1Cje at birth showed that only triplicated genes were dysregulated (Amano *et al.* 2004). This clearly demonstrates that, if one looks at a very heterogeneous population of cells in the brain, the only differentially expressed genes that will be detected are the triplicated genes. Working on a substructure such as cerebellum is one step beyond in terms of diminishing cellular complexity and shows that it is possible to detect differentially expressed euploid genes in trisomic animals.

Of course, the limitation of the study is that we observe only what we can look at, meaning that the Affymetrix chip is not exhaustive and does not contain all triplicated genes, for example.

Finally, from our study we conclude that most if not all triplicated genes are, as expected, over expressed by a factor close to 1.5 (3/2). This primary gene-dosage effect impairs about 5% of the transcriptome (secondary effect), which is five times less than what occurs during post-natal development of the cerebellum (25% change in the transcriptome). Only a small proportion of euploid genes are differentially expressed in trisomic animals at the three stages (six over expressed and four down-regulated), thus making the identification of candidate gene more difficult. Nevertheless, the list of genes involved in post-natal development of the cerebellum and dysregulated in a similar or opposite manner in trisomic animals is very short. It contains only three triplicated genes. Those three genes (*Dscr1*, *Son* and *Hmg14*) are therefore candidate genes for the snowballing or the cascading effects, leading to the phenotype observed in the cerebellum of these mice. To test this hypothesis, one way would be to try to correct the cerebellar phenotype at birth by diminishing the level of expression of the triplicated candidate genes by injection of siRNA during embryonic

development, or to create a triple transgenic mouse line over expressing the three genes and investigate the phenotype.

Acknowledgements

This work was supported by the Centre National de la Recherche Scientifique, EEC grant 00816, the Fondation Jérôme Lejeune France, the Ministère de la Recherche and the City of Paris. RXM is supported by NHMRC, Australia, and the Fondation Jérôme Lejeune France.

Supplementary Material

The following material is available for this paper online.

Table S1 Sheet 1: list of genes significantly differentially expressed between trisomic and control cerebellum both at P0, P15 and P30. Sheet 2: list of genes inversely regulated (INV) between P0 and P15 and between P0 and P30.

Table S2 Lists of differentially expressed and similarly regulated (SIM) genes [increased (+) or decreased (–)] in the cerebellum during post-natal development and in trisomy at P0, P15 and P30.

Table S3 Lists of differentially expressed and inversely regulated (INV) genes [increased (+) or decreased (–)] in the cerebellum during post-natal development and in trisomy at P0, P15 and P30.

This material is available as part of the online article from http://www.blackwell-synergy.com

References

Amano K., Sago H., Uchikawa C., Suzuki T., Kotliarova S. E., Nukina N. and Epstein C. J. and. Yamakawa K. (2004) Dosage-dependent over-expression of genes in the trisomic region of Ts1Cje mouse model for Down's syndrome. *Hum. Mol. Genet.* **13**, 1333–1340.

Antonarakis S. E., Lyle R., Dermitzakis E. T., Reymond A. and Deutsch S. (2004) Chromosome 21 and Down's syndrome: from genomics to pathophysiology. *Nat. Rev. Genet.* **5**, 1–14.

Baxter L. L., Moran T. H., Richtsmeier J. T., Troncoso J. and Reeves R. H. (2000) Discovery and genetic localization of Down's syndrome cerebellar phenotypes using the Ts65Dn mouse. *Hum. Mol. Genet.* **9**, 195–202.

Chrast R., Scott H. S., Papasavvas M. P. *et al.* (2000) The mouse brain transcriptome by SAGE: differences in gene expression between P30 brains of the partial trisomy 16 mouse model of Down's syndrome (Ts65Dn) and normals. *Genome Res.* **10**, 2006–2021.

Dauphinot L., Lyle R., Rivals I. *et al.* (2005) The cerebellar transcriptome during post-natal development of the Ts1Cje mouse, a segmental trisomy model for Down's syndrome. *Hum. Mol. Genet.* **14**, 373–384.

Epstein C. J., Korenberg J. R., Anneren G. *et al.* (1991) Protocols to establish genotype-phenotype correlations in Down syndrome. *Am. J. Hum. Genet.* **49**, 207–235.

FitzPatrick D. (2005) Transcriptional consequences of autosomal trisomy: primary gene dosage with complex downstream effects. *Trends Genet.* **21**, 249–253.

FitzPatrick D. R., Ramsay J., McGill N. I., Shade M., Carothers A. D. and Hastie N. D. (2002) Transcriptome analysis of human autosomal trisomy. *Hum. Mol. Genet.* **11**, 3249–3256.

Fuentes J. J., Pritchard M. A., Planas A. M., Bosch A., Ferrer I. and Estivill X. (1995) A new human gene from the Down's syndrome critical region encodes a proline-rich protein highly expressed in fetal brain and heart. *Hum. Mol. Genet.* **4**, 1935–1944.

Gardiner K., Fortna A., Bechtel L. and Davisson M. T. (2003) Mouse models of Down's syndrome: how useful can they be? Comparison of the gene content of human chromosome 21 with orthologous mouse genomic regions. *Gene* **318**, 137–147.

Kahlem P., Sultan M., Herwig R. *et al.* (2004) Transcript level alterations reflect gene dosage effects across multiple tissues in a mouse model of Down's syndrome. *Genome Res.* **14**, 1258–1267.

Lejeune J., Gauthier M. and Turpin R. (1959) Etude des chromosomes somatiques de neuf enfants mongoliens. *Comptes Rendus Acad. Sci.* **248**, 1721–1722.

Lim J.-H., Catez F., Birger Y., West K. L., Prymakowska-Bosak M., Postnikov Y. V. and Bustin M. (2004) Chromosomal protein HMGN1 modulates histone H3 phosphorylation. *Mol. Cell* **15**, 573–584.

Lyle R., Gehrig C., Neergaard-Henrichsen C., Deutsch S. and Antonarakis S. E. (2004) Gene expression from the aneuploid chromosome in a trisomy mouse model of Down's syndrome. *Genome Res.* **14**, 1268–1274.

Mao R., Zielke C. L., Zielke H. R. and Pevsner J. (2003) Global up-regulation of chromosome 21 gene expression in the developing Down's syndrome brain. *Genomics* **81**, 457–467.

Olson L. E., Roper R. J., Baxter L. L., Carlso N. E. J., Epstein C. J. and Reeves R. H. (2004) Down's syndrome mouse models Ts65Dn, Ts1Cje, and Ms1Cje/Ts65Dn exhibit variable severity of cerebellar phenotypes. *Dev. Dyn.* **230**, 581–589.

Reeves R. H., Irving N. G., Moran T. H., Wohn A., Kitt C., Sisodia S. S., Schmidt C., Bronson R. T. and Davisson M. T. (1995) A mouse model for Down's syndrome exhibits learning and behaviour deficits. *Nat. Genet.* **11**, 177–184.

Ryeom S., Greenwald R. J., Sharpe A. H. and McKeon F. (2003) The threshold pattern of calcinauerin-dependent gene expression is altered by loss of the endogenous inhibitor calcipressin. *Nat. Immunol.* **4**, 874–881.

Sago H., Carlson E. J., Smith D. J., Kilbridge J., Rubin E. M., Mobley W. C., Epstein C. J. and Huang T. T. (1998) Ts1Cje, a partial trisomy 16 mouse model for Down's syndrome, exhibits learning and behavioral abnormalities. *Proc. Natl Acad. Sci. USA* **95**, 6256–6261.

Saran N. G., Pletcher M. T., Natale J. E., Cheng Y. and Reeves R. H. (2003) Global disruption of the cerebellar transcriptome in a Down's syndrome mouse model. *Hum. Mol. Genet.* **12**, 2013–2019.

Sarraf S. A. and Stancheva I. (2004) Methyl-CpG binding protein MBD1 couples histone H3 methylation at lysine 9 by SETDB1 to DNA replication and chromatine assembly. *Mol. Cell* **27**, 595–605.

West K. L. (2004) HMGN proteins play roles in DNA repair and gene expression in mammalian cells. *Biochem. Soc. Trans.* **32**, 918–919.

Wynn S. L., Fisher R. A., Pagel C., Liu Q. Y., Khan I. M., Zammit P., Mazrani W., Kessling A., Lee J. S. and Buluwela L. (2000) Organization and conservation of the GART/SON/DONSON locus in mouse and human genomes. *Genomics* **68**, 57–62.

