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Gene expression profiling and qRT-PCR expression of *RRP1B*, *PCNT*, *KIF21A* and *ADRB2* in leucocytes of Down's syndrome subjects

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Introduction

Down's syndrome (DS) is one of the most common numerical chromosomal aberrations in humans, usually caused by trisomy of chromosome 21, and is the most frequent genetic cause of mental retardation. This disorder affects around 1 in 800 live births in humans, and it is caused by a complete, or occasionally partial, triplication of chromosome 21 resulting in a complex and variable phenotype (Capone 2001). The disorder is primarily characterized by cognitive and language dysfunction coupled with sensory and neuromotor deficits and a neuropathology primarily characterized by decreased brain size (Capone 2001; Mao *et al.* 2003). DS individuals are likely to suffer from a broad range of symptoms outside the nervous system, including abnormal craniofacial development, immune defects (de Hingh *et al.* 2005) and congenital heart problems (Costa *et al.* 2011). The developmental instability theory suggests that dosage imbalance on chromosome 21 as a whole disrupts diverse developmental pathways (Shapiro 1997), while the gene-dosage hypothesis suggests that dosage increase for specific genes on chromosome 21 contributes more directly to different aspects of the disease phenotype (Shapiro 1997). Recently, gene expression profiling of brain and other tissues from human subjects with trisomy 21 were performed. A small number of gene expression studies using tissues from DS subjects have been conducted, profiling fibroblasts (Li *et al.* 2006), whole blood (Smyth 2004; Tang *et al.* 2004), and amniocytes (Altug-Teber *et al.* 2007). These studies have established an important gene dosage effect for chromosome 21 but it remains unclear whether there are pervasive secondary transcriptional effects throughout the genome involved. The regulation and expression of chromosome 21 genes is likely to be both dynamic and complex (Birchler *et al.* 2001), and thus it is important that further global gene expression studies with high statistical power are performed to fully characterize the DS transcriptome.

In this study we used microarray methodology to investigate the effect of trisomy 21 on the whole gene expression set of leucocytes of peripheral blood and the results obtained in DS subjects compared with the normal population were validated through quantitative real-time PCR (qRT-PCR).

Materials and methods

Patients and controls

For microarray expression analysis, a total of 11 subjects were enrolled at the Unit of Pediatrics and Medical Genetics of the IRCCS Oasi Institute, Troina (Italy), a specialized centre for patients with intellective disability coming

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mainly from Sicily. They included: six DS patients (two males and four females) with a mean age of 49.16 ± 6.65 (range 44–56 years) and five normal subjects (two males and three females) with a mean age of 47.80 ± 5.54 (range 41–56 years). The DS cases and controls were recruited after family and/or personal informed consent.

RNA extraction from blood and preparation of RNA

Total RNA was extracted from peripheral blood leucocytes following the double extraction protocol: RNA isolation by acid guanidinium thiocyanate – phenol – chloroform extraction (Trizol Invitrogen, Paisley, UK) followed by a Qiagen (Germantown, UK) RNeasy clean-up procedure. RNA quality was assessed using Nanodrop and the Bioanalyzer (Agilent Technologies, Santa Clara, USA) (Reeves *et al.* 2001). cRNA synthesis was carried out using 300 ng of total RNA and the MessageAmp III RNA Amplification kit of total RNA (Ambion, Paisley, UK).

Array gene profiling and analysis

Fragmented cRNAs were hybridized to HG-U133 Plus 2.0 GeneChip (Affymetrix, Santa Clara, USA) following standard procedures. Data handling was mainly done using Bioconductor 2.6 (Gentleman *et al.* 2004). The robust multichip average (RMA) (Irizarry *et al.* 2003) method was employed to calculate probe set intensity. The dataset were further adjusted using the parametric empirical Bayesian method (Johnson *et al.* 2007) to eliminate batch effect caused by different temporal hybridization of arrays (Pelizzola *et al.* 2006). A data filter based on a inter-quantile range (IQR) selected in this analysis greater than 0.2 was applied. The identification of differentially expressed genes were addressed using linear models for microarray data (LIMMA) (Smyth 2004). The identification of differentially expressed genes (DEGs) were addressed using linear modelling approach and empirical Bayes methods together with false discovery rate correction of the *P* value (Johnson *et al.* 2007). The corrected *P* value that has been selected in the analysis is less or equal to 0.05 with a fold-change cutoff higher than or equal to 1. Differentially expressed genes were clustered using the PAM-clustering method (partitioning around medoid, Smyth 2004). The two gene clusters (upregulated and downregulated genes) were selected and evaluated for functional enrichment using Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.kegg.jp) sources. For each cluster, *P* value of each KEGG entry was calculated from a hypergeometric test (Birchler *et al.* 2001); *P* values lower than 0.00001 were considered significant.

Quantitative real-time PCR expression (qRT-PCR)

To validate the results obtained in the above described array analysis of DS subjects, we compared qRT-PCR in 12 DS patients (five males and seven females) with a mean age of 38.44 ± 10.51 (range 20–55 years) and 12 normal subjects (five males and seven females) with a mean age of 38.33 ± 11.28 (range 19–55 years). In this second step, the subjects studied in the microarray expression analysis were included. Once again DS cases and controls were recruited after family and/or personal informed consent. RNA extraction from leucocytes of peripheral blood was performed using RNeasy Mini Handbook (Qiagen Sciences, Germantown, USA), following the manufacturer's protocol. Quantitative RT-PCR of DS subjects was compared with controls, as previously reported by Salemi *et al.* (2011). Relative quantification analysis data were played using the comparative ΔΔCt method included in the Software v1.5 supplied with the LightCycler 480. Genes expression level was normalized to *GAPDH* level and target mean Cp (crossing point, cycle number at detection threshold) definition was used to indicate the mean normalized cycle threshold.

Results and discussion

Samples from patients with DS and normal controls were examined in our microarray analysis. A filter based on a IQR value of 0.2 eliminated 30.862 probe sets of the 54.675 present on the HGU-133 Plus 2.0 array. The remaining 23,813 probe sets were used for the identification of the DEGs using LIMMA (Smyth 2004). GenBank and UniGenes assignments were found to be significantly upregulated in 357 probe sets and downregulated in 329 ($P < 0.05$; fold change (FC) > 1) in DS samples compared to controls (data not shown). In particular, these results indicated that 43 genes localized to chromosome 21 were upregulated in the DS patients analysed ($P < 0.05$; FC > 1) (table 1). KEGG pathways that are enriched from the two gene clusters showed the most significantly upregulated and downregulated processes; these data are broadly categorized in table 2. The high sensitivity of qRT-PCR for assay of gene expression is required for validation of microarray results. For this reason we chose four genes that were found upregulated in DS patient: ribosomal RNA processing 1 homolog B (*RRP1B*) (MIM 610653) mapped to chromosome 21q23.3, pericentrin (*PCNT*) (MIM 605925) mapped to chromosome 21q22.3 both inside the minimal critical region for DS; kinesin family member 21A (*KIF21A*) (MIM 608283) mapped on chromosome 12q12; beta-2-adrenergic receptor (*ADRB2*) (MIM 109690) mapped on chromosome 5q31-q32. Each of the four genes showed significant upregulation by microarray data (table 3). *RRP1B* and *PCNT* genes were chosen primarily for their location in the minimal critical region for DS on chromosome 21. All the four genes studied by qRT-PCR plays an important role in the apoptotic and proliferative processes also involved in the early ageing of subjects with DS. In addition, the same genes were linked to diseases such as cardiomyopathy, immune-related disturbances and asthma, which have a higher incidence in the population of subjects with DS. In our case–control study with

Table 1. Genes localized to chromosome 21 upregulated in DS.

Gene symbol	Gene name	Entrez gene ID	Cytoband	*P* value	FC
AGPAT3	1-Acylglycerol-3-phosphate O-Acyltransferase 3	56894	21q22.3	0.038129152	1.492900609
APP	Amyloid beta (A4) precursor protein	351	21q21.2	0.049030455	1.363425926
C21orf2	Chromosome 21 open reading frame 2	755	21q22.3	0.023273242	1.4875
C21orf45	Chromosome 21 open reading frame 45	54069	21q22.11	0.012453394	1.438202247
C21orf59	Chromosome 21 open reading frame 59	56683	21q22.1	0.011986966	1.429967427
C21orf63	Chromosome 21 open reading frame 63	59271	21q22.11	0.015491426	1.979452055
C21orf91	Chromosome 21 open reading frame 91	54149	21q21.1	0.035261012	1.475409836
C21orf96	Chromosome 21 open reading frame 96	80215	21q22.12	0.023139283	1.793103448
CBR1	Carbonyl reductase 1	873	21q22.13	0.043506766	1.327956989
CYYR1	Cysteine/tyrosine-rich 1	116159	21q21.2	0.021022927	1.709677419
DSCR3	Down's syndrome critical region gene 3	10311	21q22.2	0.00588535	1.5
DYRK1A	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	1859	21q22.13	0.024022373	1.31824417
GART	Phosphoribosylglycinamide formyltransferase,	2618	21q22.1	0.011582698	1.546875
LSS	Lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	4047	21q22.3	0.00588535	1.9232
MCM3AP	Minichromosome maintenance complex component 3 associated protein	8888	21q22.3	0.006803713	1.474012474
MCM3APAS	MCM3AP antisense RNA (nonprotein coding)	114044	21q22.3	0.011764775	1.64
MRPS6	Mitochondrial ribosomal protein S6	64968	21q22.11	0.008262139	1.495908347
MX1	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78	4599	21q22.3	0.002591462	1.939726027
MX2	Myxovirus (influenza virus) resistance 2	4600	21q22.3	0.029481563	1.528378378
PCNT	Pericentrin	5116	21q22.3	0.023456971	1.526104418
PDXK	Pyridoxal (pyridoxine, vitamin B6) kinase	8566	21q22.3	0.00501314	1.458333333
PFKL	Phosphofructokinase, liver	5211	21q22.3	0.037129548	1.33411215
PKNOX1	PBX/knotted 1 homeobox 1	5316	21q22.3	0.042650472	1.299319728
POFUT2	Protein O-fucosyltransferase 2	23275	21q22.3	0.010958267	1.554621849
PRMT2	Protein arginine methyltransferase 2	3275	21q22.3	0.030321974	1.661363636
PSMG1	Proteasome (prosome, macropain) assembly chaperone 1	8624	21q22.3	0.004515766	1.526104418
PTTG1IP	Pituitary tumour-transforming 1 interacting protein	754	21q22.3	0.009908009	1.492830807
PWP2	PWP2 periodic tryptophan protein homolog (yeast)	5822	21q22.3	0.0101849	1.492723493
RCAN1	Regulator of calcineurin 1	1827	21q22.1-q22.2	0.019984017	1.459649123
RNF160	Ring finger protein 160	26046	21q22.11	0.036298229	1.363636364
RRP1B	Ribosomal RNA processing 1 homolog B	23076	21q22.3	0.009412083	1.458285714
RUNX1	Runt-related transcription factor 1	861	21q22.3	0.037814068	1.479166667
RWDD2B	RWD domain containing 2B	10069	21q22.11	0.028390931	1.406091371
SETD4	SET domain containing 4	54093	21q22.13	0.008985736	1.401869159
SFRS15	Splicing factor, arginine/serine-rich 15	57466	21q22.1	0.032432275	1.643312102
SON	SON DNA binding protein	6651	21q22.1-q22.2	0.026007299	1.494365685
SYNJ1	Synaptojanin 1	8867	21q22.2	0.041704359	1.453804348
TMEM50B	Transmembrane protein 50B	757	21q22.11	0.002591462	1.536804309
TMPRSS3	Transmembrane protease, serine 3	64699	21q22.3	0.046675999	1.569620253
TRAPPC10	Trafficking protein particle complex 10	7109	21q22.3	0.03820853	1.454162276
U2AF1	U2 small nuclear RNA auxiliary factor 1	7307	21q22.3	0.01121242	1.385848728
USP25	Ubiquitin specific peptidase 25	29761	21q11.2	0.017504696	1.285012285
WDR4	WD repeat domain 4	10785	21q22.3	0.030321974	1.400985222
WRB	Tryptophan rich basic protein	7485	21q22.3	0.018739779	1.553459119

FC, fold change.

qRT-PCR we have used all cases and controls separately and specifically we obtained a mean FC and standard deviation in brackets of: 1.735 (±1.088) for *RRP1B* gene, 1.678 (±0.650) for *PCNT* gene, 1.725 (±1.421) for *KIF21A* gene and 1.488 (±0.440) for *ADRB2* gene. The mean was obtained from data normalized ratio of each case–control study (12 cases versus 12 controls), according to ΔΔCt method. Costa *et al.* (2011) in a study on transcriptome in human trisomy 21, observed a particular enrichment for gene ontology (GO) terms related to immune and inflammatory responses, cell adhesion and chemokine/cytokine receptor activities. Also, Lockstone *et al.* (2007) obtained evidence of an upregulation of genes related to specific biological processes in the brain of subjects with DS; in particular some of these biological processes were: antigen presentation, antigen processing, immune response, cell migration, organogenesis,

Table 2. Biological processes altered in Down's syndrome subjects, functional enrichments with KEGG.

Id	Description	ID on list	ID on chip	*P* value
Biological processes upregulated in Down's syndrome				
05340	Primary immunodeficiency	5	29	0.000155
03010	Ribosome	4	33	0.00292
04662	B cell receptor signalling pathway	5	57	0.00368
04672	Intestinal immune network for IgA production	4	37	0.00446
05310	Asthma	3	21	0.00635
04640	Haematopoietic cell lineage	5	70	0.00887
05330	Allograft rejection	3	24	0.00929
05320	Autoimmune thyroid disease	3	26	0.0116
00290	Valine, leucine and isoleucine biosynthesis	1	2	0.0368
04020	Calcium signalling pathway	5	101	0.0376
04730	Long-term depression	3	42	0.0417
05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	3	44	0.0469
05416	Viral myocarditis	3	45	0.0496
04514	Cell adhesion molecules (CAMs)	4	82	0.064
04940	Type I diabetes mellitus	2	28	0.0941
05332	Graft versus host disease	2	28	0.0941
05322	Systematic lupus erythematosus	3	71	0.143
00630	Glyoxylate and dicarboxylate metabolism	1	9	0.155
04360	Axon guidance	3	74	0.157
00770	Pantothenate and CoA biosynthesis	1	10	0.171
04950	Maturity onset diabetes of the young	1	10	0.171
00564	Glycerophospholipid metabolism	2	44	0.196
00670	One carbon pool by folate	1	12	0.202
04060	Cytokine–cytokine receptor interaction	5	169	0.202
04142	Lysosome	3	84	0.203
04612	Antigen processing and presentation	2	52	0.251
Biological processes downregulated in Down's syndrome				
04514	Cell adhesion molecules (CAMs)	6	82	0.0102
05330	Allograft rejection	3	24	0.0164
05320	Autoimmune thyroid disease	3	26	0.0204
04080	Neuroactive ligand–receptor interaction	7	123	0.0208
04940	Type I diabetes mellitus	3	28	0.0249
05332	Graft-versus-host disease	3	28	0.0249
05220	Chronic myeloid leukaemia	4	50	0.0262
05142	Chagas disease	5	82	0.0379
00532	Glycosaminoglycan biosynthesis-chondroitin sulphate	2	14	0.0393
01040	Biosynthesis of unsaturated fatty acids	2	15	0.0447
04610	Complement and coagulation cascades	3	41	0.0658
04810	Regulation of actin cytoskeleton	6	132	0.0788
00750	Vitamin B6 metabolism	1	4	0.0884
04070	Phosphatidylinositol signalling system	3	48	0.0955
05218	Melanoma	3	48	0.0955
05212	Pancreatic cancer	3	50	0.105
05222	Small cell lung cancer	3	52	0.114
02010	ABC transporter	2	26	0.118
04062	Chemokine signalling pathway	5	116	0.124
04110	Cell cycle	4	86	0.132
04650	Natural killer cell mediated cytotoxicity	4	86	0.132
05215	Prostate cancer	3	59	0.151
04144	Endocytosis	5	125	0.155
05219	Bladder cancer	2	31	0.157
00562	Inositol phosphate metabolism	2	33	0.173
04210	Apoptosis	3	64	0.179

ID on list, number of genes involved; ID on chip, number of probe sets on the chip.

intracellular protein transport, cell cycle and response to oxidative stress. Malagó *et al.* (2005) identified significant changes in the expression pattern of DS leucocytes compared with normal subjects including key regulators of growth and proliferation and ribosomal proteins. The data obtained from our study are partially concordant with the previous

Table 3. Upregulated genes selected for validation of microarray results with qRT-PCR.

Gene symbol	Gene name	Entrez gene ID	Cytoband	*P* value*	FC*
ADRB2	Adrenergic, beta-2-, receptor, surface	1540	5q31-q32	0.003482	2.375
KIF21A	Kinesin family member 21A	55605	12q12	0.010985	1.693
PCNT	Pericentrin	5116	21q22.3	0.023456	1.526
RRP1B	Ribosomal RNA processing 1 homolog B	23076	21q22.3	0.009412	1.458

**P* value and FC refer to microarray analysis expression.

observations, confirming the importance of the inflammatory and immune-related changes seen in patients with DS. Particularly interesting, from our point of view, are the results obtained in the present study with microarray analysis and subsequent qRT-PCR validation for *RRP1B, PCNT, KIF21A* and *ADRB2* : *RRP1B* code for a protein involved in ribosomal biogenesis localized to the nucleolus, but recent data have shown that *RRP1B* also acts in suppression of metastasis, and a gene expression profile obtained following its overexpression predicted survival in breast cancers; in addition, there are evidences that *RRP1B* is important for regulation of apoptosis induced by DNA damage (Paik *et al.* 2010). *PCNT* is a very large coiled-coil protein that localizes to centrosomes throughout the cell cycle (Flory *et al.* 2000). The centrosome is a cell component that organizes cytoplasmic organelles and primary cilia in interphase cells, and mitotic spindle microtubules to ensure proper chromosome segregation during cell division. Depletion of *PCNT* by a small interfering RNA lead to programmed cell death (apoptosis) after activation of mitotic checkpoints and arrest of cells in the transition from G2 phase to mitosis (Zimmerman *et al.* 2004). In addition, *PCNT* mutations are associated with a type of primordial dwarfism, intrauterine growth retardation, cardiomyopathy and early onset type 2 diabetes (Rauch 2011). *KIF21A* is a member of the kinesin superfamily, a large gene family of microtubule-dependent motors with 45 members in humans involved in the anterograde fast axonal transport (Vale *et al.* 1985). Specifically, *KIF21A* is expressed mostly in the juvenile brain and the protein is present throughout the neuron (Marszalek *et al.* 1999). *ADRB2* gene, besides being associated with asthma-related mechanisms (Litonjua 2006), is involved in the carcinogenic processes; basically, it seems to be related with cell proliferation, apoptosis, chemotaxis, development of metastasis, tumour growth, and angiogenesis (Perez-Sayans *et al.* 2011). The expression data obtained in this study for *RRP1B*, *PCNT* and *ADRB2* underline the potential role that could have apoptosis in term of premature ageing, cerebral involution, inflammatory and immune-related biological processes in DS. In addition, PCNT abnormal protein translation could probably play a role in mitotic stages of embryonic development of DS subjects. From another point of view, *PCNT* abnormal expression or its mutations could affect cardiomyopathy and early onset type 2 diabetes present in DS patients. Moreover, changes in the expression levels of *KIF21A* may affect the axonal transport and the development of the nervous system with, once again, strong implications on the resulting phenotype of the DS subjects. This study offers an insight into transcriptional changes in DS leucocytes and indicates that four candidate genes (*RRP1B, PCNT, KIF21A* and *ADRB2)* are possibly deeply involved into the molecular mechanism of DS pathology and worthy of further investigations. Our observations are clearly limited by the small numbers of individuals analysed but the results obtained largely confirm previous studies. Together, these evidences indicate that the same alterations of many biological processes involved in DS are present in various tissues.

Larger-scale association studies are necessary to confirm our findings regarding *RRP1B, PCNT, KIF21A* and *ADRB2*. In any case it seems appropriate to investigate the expression of these genes in the context of the whole genome.

References

Altug-Teber O., Bonin M., Walter M., Mau-Holzmann U. A., Dufke A., Stappert H. *et al.* 2007 Specific transcriptional changes in human fetuses with autosomal trisomies. *Cytogenet. Genome Res.* **119**, 171–184.

Birchler J. A., Bhadra U., Bhadra M. P. and Auger D. L. 2001 Dosage-dependent gene regulation in multicellular eukaryotes: implications for dosage compensation, aneuploid syndromes, and quantitative traits. *Dev. Biol.* **234**, 275–288.

Capone G. T. 2001 Down syndrome: advances in molecular biology and the neurosciences. *J. Dev. Behav. Pediatr.* **22**, 40–59.

Costa V., Angelini C., D'Apice L., Mutarelli M., Casamassimi A., Sommese L. *et al*. 2011 Massive-scale RNA-seq analysis of non ribosomal transcriptome in human trisomy 21. *PLoS ONE* **6**, e18493.

de Hingh Y. C., van der Vossen P. W., Gemen E. F., Mulder A. B., Hop W. C., Brus F. and de Vries E. 2005 Intrinsic abnormalities of lymphocyte counts in children with down syndrome. *J. Pediatr.* **147**, 744–747.

Flory M. R., Moser M. J., Monnat Jr R. J. and Davis T. N. 2000 Identification of a human centrosomal calmodulin-binding protein that shares homology with pericentrin. *Proc. Natl. Acad. Sci. USA* **7**, 5919–5923.

Gentleman R. C., Carey V. J., Bates D. M., Bolstad B., Dettling M., Dudoit S. *et al*. 2004 Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* **5**, R80.

Irizarry R. A., Bolstad B. M., Collin F., Cope L. M., Hobbs B. and Speed T. P. 2003 Summaries of affymetrix GeneChip probe level data. *Nucleic Acids Res.* **31**, e15.

Johnson W. E., Rabinovic A. and Li C. 2007 Adjusting batch effects in microaaray expression data using Empirical Bayes methods. *Biostatistics* **8**, 118–127.

Li C. M., Guo M., Salas M., Schupf N., Silverman W., Zigman W. B. *et al*. 2006 Cell type-specific over-expression of chromosome 21 genes in fibroblasts and fetal hearts with trisomy 21. *BMC Med. Genet.* **7**, 24.

Litonjua A. A. 2006 The significance of beta2-adrenergic receptor polymorphisms in asthma. *Curr. Opin. Pulm. Med.* **12**, 12–17.

Lockstone H. E., Harris L. W., Swatton J. E., Wayland M. T., Holland A. J. and Bahn S. 2007 Gene expression profiling in the adult Down syndrome brain. *Genomics* **90**, 647–660.

Malago Jr W., Sommer C. A., Del Cistia Andrade C., Soares-Costa A., Abrao Possik P., Cassago A. *et al*. 2005 Gene expression profile of human Down syndrome leukocytes. *Croat. Med. J.* **46**, 647–656.

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

Marszalek J. R., Weiner J. A., Farlow S. J., Chun J. and Goldstein L. S. 1999 Novel dendritic kinesin sorting identified by different process targeting of two related kinesins: KIF21A and KIF21B. *J. Cell Biol.* **145**, 469–479.

Paik J. C., Wang B., Liu K., Lue J. K. and Lin W. C. 2010 Regulation of E2F1-induced apoptosis by the nucleolar protein RRP1B. *J. Biol. Chem.* **285**, 6348–6363.

Pelizzola M., Pavelka N., Foti M. and Ricciardi-Castagnoli P. 2006 AMDA: an R package for the automated microarray data analysis. *BMC Bioinformatics* **7**, 335.

Perez-Sayans M., Somoza-Martin J. M., Barros-Angueira F., Diz P. G., Gandara Rey J. M. and Garcia-Garcia A. 2011 Beta-adrenergic receptors in cancer: therapeutic implications. *Oncol. Res.* **19**, 45–54.

Rauch A. 2011 The shortest of the short: pericentrin mutations and beyond. *Best. Pract. Res. Clin. Endocrinol. Metab.* **25**, 125–130.

Reeves R. H., Baxter L. L. and Richtsmeier J. T. 2001 Too much of a good thing: mechanisms of gene action in Down syndrome. *Trends Genet.* **17**, 83–88.

Salemi M., Barone C., Romano C., Ridolfo F., Gulotta E., Scavuzzo C. *et al*. 2011 Differential expression of PARP1 mRNA in leucocytes of patients with Down syndrome. *J. Genet.* **90**, 469–472.

Shapiro B. L. 1997 Whither Down syndrome critical regions? *Hum. Genet.* **99**, 421–423.

Smyth G. K. 2004 Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat. Appl. Genet. Mol. Biol.* **3**, article 3.

Tang Y., Schapiro M. B., Franz D. N., Patterson B. J., Hickey F. J., Schorry E. K. *et al*. 2004 Blood expression profiles for tuberous sclerosis complex 2, neurofibromatosis type 1, and Down's syndrome. *Ann. Neurol.* **56**, 808–814.

Vale R. D., Reese T. S. and Sheetz M. P. 1985 Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* **42**, 39–50.

Zimmerman W. C., Sillibourne J., Rosa J. and Doxsey S. J. 2004 Mitosis-specific anchoring of gamma tubulin complexes by pericentrin controls spindle organization and mitotic entry. *Mol. Biol. Cell* **15**, 3642–3657.

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