

Protein levels of genes encoded on chromosome 21 in fetal Down syndrome brain: Challenging the gene dosage effect hypothesis (Part III)

M. S. Cheon¹, S. H. Kim¹, V. Ovod², N. Kopitar Jerala³, J. I. Morgan⁴, Y. Hatefi⁵, T. Ijuin⁶,
T. Takenawa⁶, and G. Lubec¹

¹Department of Pediatrics, University of Vienna, Vienna, Austria

²MAbs Development and Production, FIT Biotech Oyj Plc, Tampere, Finland

³Department of Biochemistry and Molecular Biology, Jozef Stefan Institute, Ljubljana, Slovenia

⁴Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, Tennessee, U.S.A.

⁵Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California, U.S.A.

⁶Department of Biochemistry, The Institute of Medical Science, The University of Tokyo, Japan

Received August 12, 2002

Accepted September 12, 2002

Published online January 30, 2003; © Springer-Verlag 2003

Summary. Down syndrome (DS) is the most frequent genetic disorder with mental retardation and caused by trisomy 21. Although the gene dosage effect hypothesis has been proposed to explain the impact of extra chromosome 21 on the pathology of DS, a series of evidence that challenge this hypothesis has been reported. The availability of the complete sequences of genes on chromosome 21 serves now as starting point to find functional information of the gene products, but information on gene products is limited so far. We therefore evaluated expression levels of six proteins whose genes are encoded on chromosome 21 (synaptojanin-1, chromosome 21 open reading frame 2, oligomycin sensitivity conferring protein, peptide 19, cystatin B and adenosine deaminase RNA-specific 2) in fetal cerebral cortex from DS and controls at 18–19 weeks of gestational age using Western blot analysis. Synaptojanin-1 and C21orf2 were increased in DS, but others were comparable between DS and controls, suggesting that the DS phenotype cannot be simply explained by gene dosage effects. We are systematically quantifying all proteins whose genes are encoded on chromosome 21 in order to provide a better understanding of the pathobiochemistry of DS at the protein level. These studies are of significance as they show for the first time protein levels that are carrying out specific function in human fetal brain with DS.

Keywords: Adenosine deaminase RNA-specific 2 – chromosome 21 – Chromosome 21 open reading frame 2 – Cystatin B – Down syndrome – Oligomycin sensitivity conferring protein – Peptide 19 – Synaptojanin-1

Abbreviations: ADAR2, adenosine deaminase RNA-specific 2; C21orf2, chromosome 21 open reading frame 2; DS, Down syndrome; NSE, neuron specific enolase; OSCP, oligomycin sensitivity conferring protein; PEP-19, peptide 19

Introduction

Down syndrome (DS), trisomy 21, is the most common genetic cause of mental retardation with a prevalence of 1:700 in live births. To explain how three copies of normal genes on chromosome 21 segment can lead to the complex metabolic and developmental aberration, the gene dosage effects hypothesis has been suggested. However, this hypothesis has been challenged by several investigators including our group (Greber-Platzer et al., 1999; Engidawork et al., 2001a). In addition, the molecular and biochemical mechanisms underlying the pathology of DS are still unknown, although analysis of the sequence of chromosome 21 has been essentially completed. This made us investigate expression levels of six proteins encoded on chromosome 21 – synaptojanin-1 (21q22.2), chromosome 21 open reading frame 2 (C21orf2; 21q22.3), oligomycin sensitivity conferring protein (OSCP; 21q22.11), peptide 19 (PEP-19; 21q22.2–22.3), cystatin B (21q22.3) and adenosine deaminase RNA-specific 2 (ADAR2; 21q22.3) (Fig. 1) – in fetal brains from DS and controls at the early second trimester.

Synaptojanin-1 is a highly abundant polyphosphoinositide phosphatase in nerve terminals and plays a role in clathrin-mediated synaptic vesicle endocytosis and synaptic transmission (Stenmark, 2000).

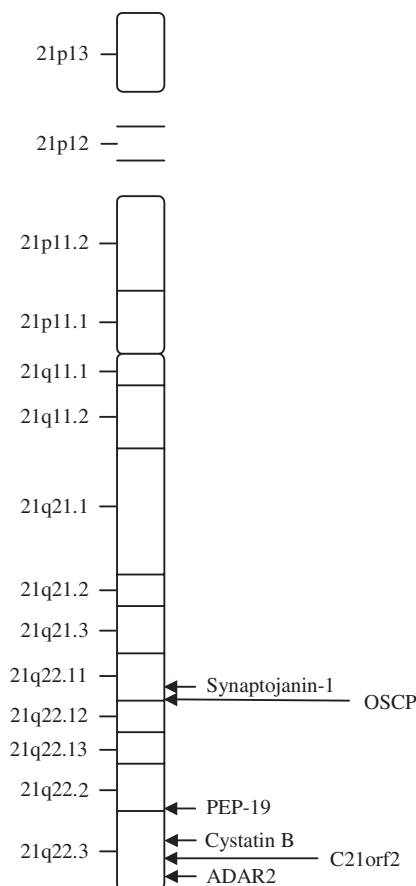


Fig. 1. Giemsa banding (G-bands) of human chromosome 21. Arrows indicate six proteins, products of genes encoded on chromosome 21, used in this study

Synaptojanin-1 is also regulating rearrangement of actin filament through action on actin-regulatory proteins (Sakisaka, 1997). C21orf2 is encoded on the terminal part of chromosome 21 and this region is known to contain yet unidentified genes linked to hereditary diseases such as autoimmune poly-endo-crinopathy candidiasis ectodermal dystrophy (Aaltonen et al., 1994) and two forms (DFNB 8/10) of non-syndromic hereditary deafness (Veske et al., 1996). The mitochondrial ATPase consists of F1, which is the catalytic segment for synthesis and hydrolysis of ATP, F0, which is the membrane integral proton translocating sector, and a structure, the stalk, which connects F1 and F0 (Walker and Collison, 1994). The stalk is made up of subunits from both F1 and F0, and OSCP is one subunit of F0 and constitutes the stalk (Belobrudov et al., 1996). PEP-19, also known as Purkinje cell protein 4 (PCP4), is a neuron-specific protein capable of binding to calmodulin and inhibiting calcium-calmodulin signaling (Slemmon et

al., 1996). This protein was originally isolated based on its developmental regulation in rat cerebellum (Ziai et al., 1986), and expression of PEP-19 has recently been shown to modulate calcium-dependent kinase activity within cells (Johanson et al., 2000). Cystatin B, also termed stefin B, interacts with and inhibits particular cysteine proteases of the cathepsin family (Turk and Bode, 1991). Loss-of-function mutations in cystatin B gene are responsible for the primary deficit in progressive myoclonus epilepsy, an autosomal recessive disorder with neurological dysfunction (Pennacchio et al., 1996). The enzyme ADAR2 (also ADARB1), a human homologue to rat RED1, is a double-stranded RNA-specific adenosine deaminase which is involved in the editing of pre-mRNA by the site-selective conversion of adenosine to inosine (Mittaz et al., 1997). Its main physiological substrates are pre-mRNAs encoding subunits of ionotropic glutamate receptors or serotonin receptors in the brain (Keller et al., 1999).

Materials and methods

Fetal brain samples

Fetal brain tissues (cerebral cortex) of DS (5 females with 18–19 weeks of gestational age) and controls (4 females with 18–19 weeks of gestational age) were used in this study. Brain samples were obtained from Dr. Mara Dierssen (Medical and Molecular Genetics Center-IRO, Hospital Duran i Reynals, Barcelona, Spain) and Dr. Joan Carles Ferreres (Department of Pathology UDIAT-CD, Corporació Sanitària Parc Tauli, Sabadell, Barcelona, Spain). All samples had a postmortem time of less than 6 hours, were stored at -70°C and the freezing chain was never interrupted until use.

Antibodies

The details of the preparation and characterization of antibodies have been described previously: synaptojanin-1 (Sakisaka et al., 1997); C21orf2 (Krohn et al., 1997); OSCP (Hekman and Hafei, 1991); PEP19 (Ziai et al., 1988); cystatin B (Kopitar-Jerala et al., 1993). Three antibodies for RED-1 (Exalpha Biologicals, Inc, USA), neuron specific enolase (NSE; Chemicon, UK), and actin (Sigma, USA), were purchased.

Western blotting

Fetal brain tissues ground under liquid nitrogen were homogenized in lysis buffer containing protease inhibitor cocktail tablet (Rache, Germany) at 4°C and centrifuged at $8,000 \times g$ for 10 minutes. The BCA protein assay kit (Pierce, USA) was applied to determine the concentration of protein in the supernatant. Samples ($10\mu\text{g}$) were mixed with the sample buffer (100 mM Tris-HCl, 2% SDS, 1% 2-mercaptoethanol, 2% glycerol, 0.01% bromophenol blue, pH 7.6), incubated at 95°C for 15 minutes and loaded onto a ExcelGel SDS homogenous gel (Amersham Pharmacia Biotech, Sweden). Electrophoresis was performed with Multiphor II Electrophoresis System (Amersham Pharmacia Biotech). Proteins separated on the

gel were transferred onto PVDF membrane (Millipore, USA) and membranes were incubated in blocking buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20 and 2% non-fat dry milk). Membranes were incubated for 2 hours at room temperature with diluted primary antibodies (1:50 for cystatin B; 1:500 for C21orf2; 1:1,000 for synaptojanin-1, RED1, and actin; 1:3,000 for NSE; 1:5,000 for PEP-19; 1:10,000 for OSCP). After 3 times washing for 15 minutes with blocking buffer, membranes were probed with secondary antibodies coupled to horseradish peroxidase (Southern Biotechnology Associates, Inc., USA) for 1 hour. Membranes were washed 3 times for 15 minutes and developed with the Western Lightning™ chemiluminescence reagents (PerkinElmer Life Sciences, Inc., USA).

Statistics

The density of immunoreactive bands was measured by RFLPscan version 2.1 software program (Scanalytics, USA). Between group differences were calculated by non-parametric Mann-Whitney U test using GraphPad Instat2 program and the level of significance was considered at $P < 0.05$.

Results

We evaluated the expression level of six proteins encoded on chromosome 21 (synaptojanin-1, C21orf2, OSCP, PEP-19, cystatin B and ADAR2) in fetal brains with DS compared to controls by Western blot analysis (Fig. 2). Two proteins, NSE and actin were used as reference proteins for neurons, and total cells, respectively, and showed comparable expression levels in DS and controls. Protein expression of synaptojanin-1 and C21orf2 was increased in fetal DS brain. The increase of synaptojanin-1 was even more pronounced when related to actin or NSE level. NSE-normalized C21orf2 level was increased in DS, and the protein levels of C21orf2 also showed an increase when normalized with those of actin, although not reaching statistical significance (Fig. 3). However, the density of immunoreactive bands of other four proteins was comparable between fetal DS and controls. When levels of four proteins were normalized with those of actin or NSE, no difference was observed between two groups.

Discussion

Synaptojanin-1 has two isoforms of the protein with molecular masses of 145 and 170 kDa and both forms have been thought to have a general function in clathrin-mediated endocytosis. It was revealed that the 145 kDa isoform could not be detected in fetal and increased dramatically in the adult period, whereas the 170 kDa isoform increased during fetal period and decreased to below detectability in the adult rat brain

(Ramjaun and McPherson, 1996). In our western blot analysis of human fetal brain, consistent with this report, the single band of 170 kDa was detected, and Arai et al. also observed the single band of 170 kDa during human fetal period (Arai et al., 2001). Synaptojanin-1 expression was observed in a variety of neuronal cells of the fetal cerebrum in both DS and controls and the temporal and spatial expression pattern of synaptojanin-1 may suggest a broader role in not only synaptic vesicle recycling, but also the regulation of neuronal migration and synaptogenesis at the stage of human fetal development when the majority of cortical migration occurs (from 14 to 20 gestational weeks). Furthermore, in comparison with controls, DS brains clearly showed higher immunoreactivity of synaptojanin-1 (Arai et al., 2002). This finding is in agreement with our present result showing increased expression levels of synaptojanin-1 in fetal DS brain. Interestingly, intersectin 1, another protein which maps to chromosome 21 and is also thought to be involved in clathrin-mediated endocytosis and synaptic vesicle recycling, was overexpressed in brains from DS patients (Pucharcos et al., 1999). Although the reason for excessive expression of synaptojanin-1 in fetal DS brains is unknown, the contribution of gene dosage effect can not be ruled out. Another possibility is that overexpression of synaptojanin-1 may be associated with mechanisms occurring in developing DS brains to compensate the decreased number of synapses and abnormal synaptic density and length. In addition, increased levels of synaptojanin-1 may affect aberrant expression/organization of actin-related proteins (Sakisaka et al., 1997) in fetal DS brain that we have demonstrated in a previous study (Weitzdoerfer et al., 2002). The most likely explanation, however, is that the increased synaptojanin 1 (a polyphosphoinositide phosphatase) reflects impaired signaling, that has been already shown to occur in DS.

Since C21orf2 was identified, this gene has been thought to be a plausible candidate for DFNB8/10 (see above; Krohn et al., 1997). Recently, however, the Tmprss3 gene on chromosome 21, which encodes a transmembrane serine protease, was found to be responsible for both the DFNB8 and DFNB10 phenotypes (Scott et al., 2001). Although immuno-fluorescence studies confirmed that the C21orf21 protein is located in the mitochondria (Krohn et al., 1997) and the mRNA and protein of this gene showed ubiquitous expression in fetal and adult tissues studied, including heart, brain, skeletal muscle

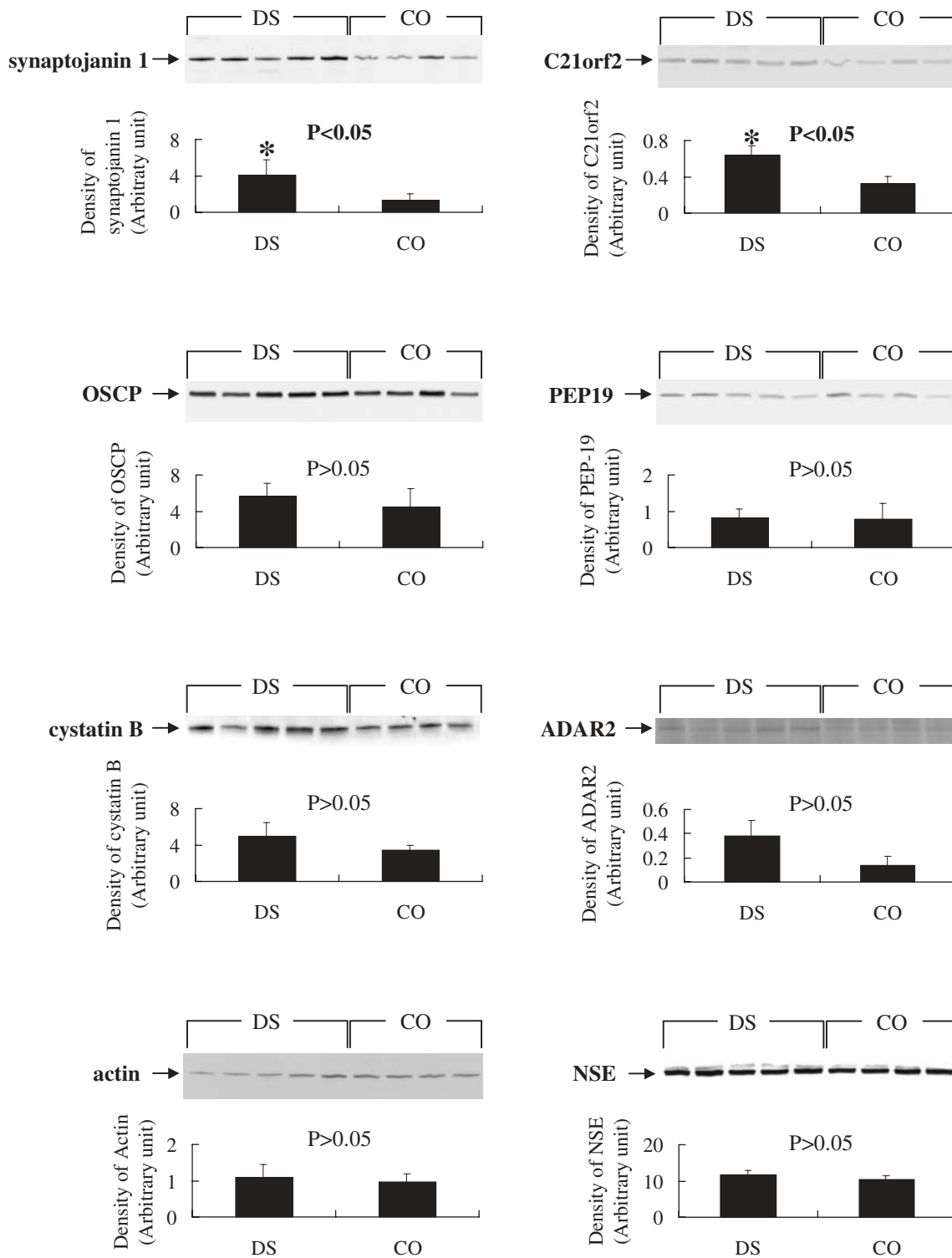


Fig. 2. Western blot analysis for six proteins whose proteins are encoded on chromosome 21 in cerebral cortex from fetal brain with DS and controls. Denatured proteins (10 μ g) were loaded, separated on a homogeneous gel and transferred onto PVDF membrane. As described in “materials and methods”, the membranes were incubated with primary and secondary antibodies, and immuno-reactive bands (synaptojanin-1, 170 kDa; cystatin B, 12 kDa; C21orf2, 30 kDa; PEP-19, 20 kDa; OSCP, 28 kDa; ADAR2, 90 kDa; NSE, 45 kDa; actin, 42 kDa) were detected using chemiluminescence reagents. The density of detected bands was measured and calculated by non-parametric Mann-Whitney U test, and the level of significance was considered at $P < 0.05$.

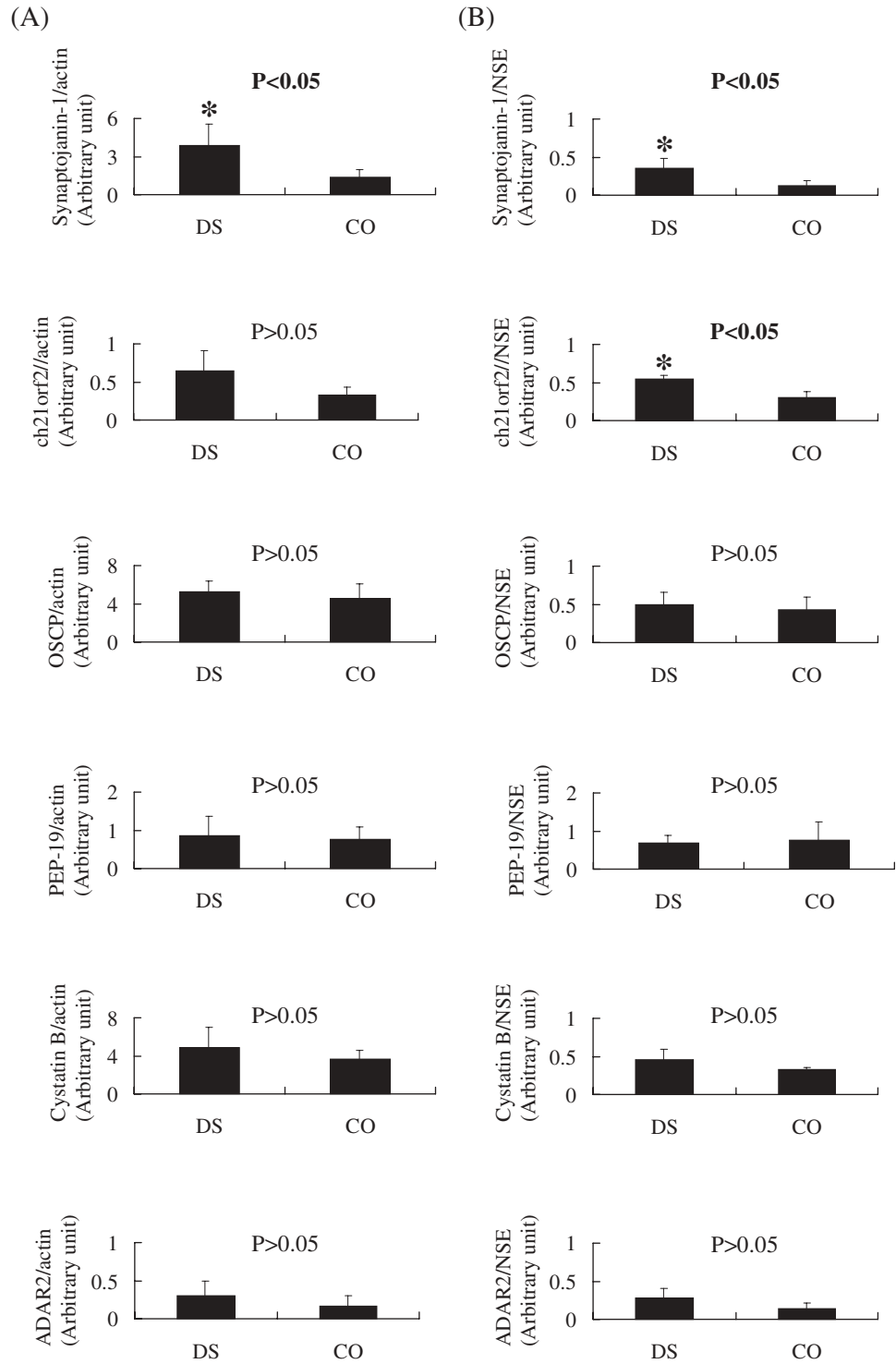


Fig. 3. Expression levels of six proteins normalized with those of actin (A) or NSE (B). The density of immunoreactive band for each protein was normalized with that of actin or NSE, which were used as reference proteins

and pancreas (Scott et al., 1998), known to be a rich source for mitochondria, the function of this protein inside mitochondria remains completely unknown so far. Based on the considerable increase of C21orf2 protein expression in fetal DS brain, C21orf2 could

play a role in the mitochondrial deficits of fetal DS brain (Kim et al., 2001).

Several observations have demonstrated that OSCP is central to correct F1 and F0 interactions and energy coupling during ATP synthesis (Joshi et al., 1996).

Interestingly, OSCP has been identified as a new estradiol binding protein and estradiol or estrogenic compounds inhibited F1F0 ATPase activity of rat brain (Zheng and Ramirez, 1999). This inhibitory property is quite similar to the endogenous F1 inhibitor protein that inhibits the ATPase activity without effect on ATP synthase activity of this enzyme. The F1 inhibitor protein has been implicated in protecting the cells from ischemic injury by inhibiting ATPase activity, therefore preserving ATP (Rouslin, 1991). Since it is most likely that the effect of estrogens on the ATPase is mediated by their binding to OSCP, OSCP per se could modulate cellular energy metabolism. In our previous study, beta subunit of ATPase was decreased in frontal cortex from DS patients (Kim et al., 2000), but alpha subunit precursor and d subunit of ATPase were not changed in fetal DS brain (Kim et al., 2001). Taken together, our present result shows unchanged protein expression of OSCP suggesting correct, structural F1 and F0 interactions and proton transport in fetal DS brains.

In our experiment, we observed that PEP-19 was expressed in cerebral cortex of fetal brain. Although PEP-19 has been thought to be a marker of the Purkinje cell, immunohistochemistry studies have demonstrated its expression in neurons of other human brain areas including basal ganglia, hippocampus and cerebellum (Utal et al., 1998). PEP-19 expression was dramatically reduced in both Huntington's and Alzheimer's disease brains (Utal et al., 1998; Slemmon et al., 1994), suggesting a potential role for this protein in the pathophysiology of neurodegenerative diseases through a mechanism of calcium-calmodulin dysregulation. However, there was no detectable change of PEP-19 immunoreactivity in adult DS brains (Utal et al., 1998). Recently, it was published that PEP-19 inhibited cell death following apoptotic stimuli at a point upstream of caspase-3 activation, and this result implicates a mechanism by which neuronal cells may modulate signal transduction pathways leading to apoptosis (Erhardt et al., 2000). Considering the unchanged levels of apoptosis-related proteins including caspase-3 in fetal DS brain (Engidawork et al., 2001b), the comparable expression of PEP19 between fetal DS and control brains could suggest that enhanced apoptosis may not be apparent during the phase of brain development of DS studied.

In addition to progressive myoclonus epilepsy, cystatin B has been implicated in the pathophysiology of some other neurodegenerative diseases. Increased

levels of cystatin B protein and presence of the cathepsins in the senile plaques have been observed in brains of patients with adult DS and Alzheimer's disease (Lemere et al., 1995). A recent in vitro study has demonstrated that cathepsin B, which is inhibited by cystatin B, induced neuronal apoptosis and caspase 3 activation (Kingham and Pocock, 2001), indicating an important role of cystatin B in blocking apoptosis. Moreover, mutant mice lacking cystatin B exhibit apoptosis of cerebellar granule cells (Pennacchio et al., 1998) and have increased expression of apoptosis and glial activation genes (Lieuallen et al., 2001). Herein, cystatin B did not change in fetal DS brain. Consistently, in our previous study, the expression of α -crystallin A subunit, whose gene is also encoded on chromosome 21 and has the ability to protect cells from apoptosis by an inhibition of caspase activity (Kamradt et al., 2001), was comparable between fetal DS and controls (Cheon et al., 2002). As discussed for PEP19, the unaltered expression of cystatin B in fetal DS brain suggests that enhanced apoptosis might not be involved in the abnormal brain development of DS.

RNA editing plays an important role in determining physiological characterization of receptor channels such as Ca^{2+} permeability, G-protein coupling and desensitization kinetics. Interestingly, glutamate receptor subunit 5, one of substrates of ADAR2, maps also to chromosome 21, providing a logical link between ADAR2 and glutamate receptor subunit 5 editing. The adenosine deaminase family members including ADAR2 are candidates for involvement in neurological disorders such as epilepsy, because of their expression patterns and described functions, and, indeed, the mutant ADAR2^{-/-} mice became prone to seizure and died early in life (Higuchi et al., 2000). It was revealed that ADAR2 is associated with production of null-function endothelin B receptor, a cause of Hirschsprung disease, by RNA editing/splicing suggesting the involvement of ADAR2 in the etiology of this heterogeneous genetic disorder (Tanoue et al., 2002). In this study, we used anti-rat RED1 antibody to detect ADAR2 protein in human fetal brain and the human enzyme is 95% identical to the rat homologue (Gerber et al., 1997). We observed relatively weak immunoreactive band for ADAR2 protein, although the transcripts of ADAR2 were proposed to be strongly expressed in human fetal and adult brains (Mittaz et al., 1997). However, ADAR2 was first detectable in the rat brain at embryonic day 19, almost at term (Paupard et al., 2000). Additionally, both

transcription and processing of *Drosophila* homologue transcripts to ADAR2 are under strict developmental control and suggest that the process of RNA editing is tightly regulated (Palladino et al., 2000). In rat brain, ADAR2 mRNA expression was regulated in a cell-specific manner throughout development (Paupard et al., 2000). The current data demonstrate that the expression of ADAR2 was comparable between fetal DS and controls and do not explain the higher basal Ca^{2+} concentration (Cardenas et al., 2002)) and impaired Ca^{2+} buffering capacity (Cardenas et al., 1999) in the mouse fetal trisomy 16 (corresponding to human chromosome 21)-derived neuronal cell lines.

Our present data showing increased levels of synaptojanin-1 and C21orf2 with unaltered expression of four proteins of chromosome 21 in fetal DS brain suggest that the gene dosage effect hypothesis is not sufficient to fully explain the DS phenotype. We are in the process of quantifying all gene products of chromosome 21 and these studies are considered of significance as they show for the first time levels of functional proteins encoded on chromosome 21 in human fetal brain with DS rather than RNA levels. Furthermore, our findings may provide the basis for better understanding of the pathobiochemistry and neuropathology of DS.

References

- Aaltonen J, Bjorses P, Sandkuijl L, Perheentupa J, Peltonen L (1994) An autosomal locus causing autoimmune disease: auto-immune polyglandular disease type I assigned to chromosome 21. *Nat Genet* 8: 83–87
- Arai Y, Ijuin T, Takenawa T, Becker LE, Takashima S (2002) Excessive expression of synaptojanin in brains with Down syndrome. *Brain Dev* 24: 67–72
- Arai Y, Ijuin T, Itoh M, Takenawa T, Takashima S, Becker LE (2001) Developmental changes of synaptojanin expression in the human cerebrum and cerebellum. *Brain Res Dev Brain Res* 129: 1–9
- Belogradov GI, Tomich JM, Hatefi Y (1996) Membrane topography and near-neighbor relationships of the mitochondrial ATP synthase subunits e, f, and g. *J Biol Chem* 271: 20340–20345
- Cardenas AM, Rodriguez MP, Cortes MP, Alvarez RM, Wei W, Rapoport SI, Shimahara T, Caviedes R, Carviedes P (1999) Calcium signals in cell lines derived from the cerebral cortex of normal and trisomy 16 mice. *Neuroreport* 10: 363–369
- Cardenas AM, Allen DD, Arriagada C, Olivares A, Bennett LB, Caviedes R, Dagnino-Subiabre A, Mendoza IE, Segura-Aguilar J, Rapoport SI, Caviedes P (2002) Establishment and characterization of immortalized neuronal cell lines derived from the spinal cord of normal and trisomy 16 fetal mice, an animal model of Down syndrome. *J Neurosci* 68: 46–58
- Cheon MS, Bajo M, Kim SH, Claudio JO, Stewart AK, Patterson D, Kruger WD, Kondoh H, Lubec G (2003) Protein levels of genes encoded on chromosome 21 in fetal Down syndrome brain: challenging the gene dosage effect hypothesis (Part II). *Amino Acids* 24: 119–125
- Engidawork E, Balic N, Fountoulakis M, Dierssen M, Greber-Platzer S, Lubec G (2001a) β -Amyloid precursor protein, ETS-2 and collagen alpha 1 (VI) chain precursor, encoded on chromosome 21, are not overexpressed in fetal Down syndrome: further evidence against gene dosage effect. *J Neural Transm [Suppl]* 61: 335–346
- Engidawork E, Balic N, Juranville JF, Fountoulakis M, Dierssen M, Lubec G (2001b) Unaltered expression of Fas (CD95/APO-1), caspase-3, bcl-2 and annexins in brain of fetal Down syndrome: evidence against increased apoptosis. *J Neural Transm [Suppl]* 61: 149–162
- Erhardt JA, Legos JJ, Johanson RA, Slemmon JR, Wang X (2000) Expression of PEP-19 inhibits apoptosis in PC12 cells. *Neuroreport* 11: 3719–3723
- Gerber A, O'Connell MA, Keller W (1997) Two forms of human double-stranded RNA-specific editase 1 (hRED1) generated by the insertion of an Alu cassette. *RNA* 3: 453–463
- Greber-Platzer S, Schatzmann-Turhani D, Cairns N, Balcz B, Lubec G (1999) Expression of the transcription factor ETS2 in brain of patients with Down syndrome-evidence against the overexpression-gene dosage hypothesis. *J Neural Transm [Suppl]* 57: 269–281
- Hekman C, Hatefi Y (1991) The F0 subunits of bovine mitochondrial ATP synthase complex: purification, antibody production, and interspecies cross-immunoreactivity. *Arch Biochem Biophys* 284: 90–97
- Higuchi M, Maas S, Single FN, Hartner J, Rozov A, Burnashev N, Feldmeyer D, Sprengel R, Seeburg PH (2000) Point mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-editing enzyme ADAR2. *Nature* 406: 78–81
- Johanson RA, Sarau HM, Foley JJ, Slemmon JR (2000) Calmodulin-binding peptide PEP-19 modulates activation of calmodulin kinase II In situ. *J Neurosci* 20: 2860–2866
- Joshi S, Cao GJ, Nath C, Shah J (1996) Oligomycin sensitivity conferring protein of mitochondrial ATP synthase: deletions in the N-terminal end cause defects in interactions with F1, while deletions in the C-terminal end cause defects in interactions with F0. *Biochemistry* 35: 12094–12103
- Kamradt MC, Chen F, Cryns VL (2001) The small heat shock protein alpha B-crystallin negatively regulates cytochrome c- and caspase-8-dependent activation of caspase-3 by inhibiting its autoproteolytic maturation. *J Biol Chem* 276: 16059–16063
- Keller W, Wolf J, Gerber A (1999) Editing of messenger RNA precursors and of tRNAs by adenosine to inosine conversion. *FEBS Lett* 452: 71–76
- Kim SH, Fountoulakis M, Dierssen M, Lubec G (2001) Decreased protein levels of complex I 30-kDa subunit in fetal Down syndrome brains. *J Neural Transm [Suppl]* 61: 109–116
- Kim SH, Vlkolinsky R, Cairns N, Lubec G (2000) Decreased levels of complex III core protein 1 and complex V β chain in brains from patients with Alzheimer's disease and Down syndrome. *Cell Mol Life Sci* 57: 1810–1816
- Kingham PJ, Pocock JM (2001) Microglial secreted cathepsin B induces neuronal apoptosis. *J Neurochem* 76: 1475–1484
- Kopitar-Jerala N, Curin-Serbec V, Jerala R, Krizaj I, Gubensek F, Turk V (1993) Monoclonal antibodies to human stefin B and determination of their epitopes. *Biochim Biophys Acta* 1164: 75–80
- Krohn K, Vladimir O, Vilja P, Heino M, Scott HS, Kyriakou DS, Antonarakis S, Jacobs HT, Isola J, Peterson P (1997) Immunochemical characterization of a novel mitochondrially located protein encoded by a nuclear gene within the DFNB8/10 critical region on 21q22.3. *Biochem Biophys Res Commun* 238: 806–810

- Lemere CA, Munger JS, Shi GP, Natkin L, Haass C, Chapman HA, Selkoe DJ (1995) The lysosomal cysteine protease, cathepsin S, is increased in Alzheimer's disease and Down syndrome brain. An immunocytochemical study. *Am J Pathol* 146: 848–860
- Lieuallen K, Pennacchio LA, Park M, Myers RM, Lennon GG (2001) Cystatin B-deficient mice have increased expression of apoptosis and glial activation genes. *Hum Mol Genet* 10: 1867–1871
- Mittaz L, Scott HS, Rossier C, Seeburg PH, Higuchi M, Antonarakis SE (1997) Cloning of a human RNA editing deaminase (ADARB1) of glutamate receptors that maps to chromosome 21q22.3. *Genomics* 41: 210–217
- Palladino MJ, Keegan LP, O'Connell MA, Reenan RA (2000) dADAR, a Drosophila double-stranded RNA-specific adenosine deaminase is highly developmentally regulated and is itself a target for RNA editing. *RNA* 6: 1004–1018
- Paupard M-C, O'Connell MA, Gerber AP, Zukin RS (2000) Patterns of developmental expression of the RNA editing enzyme rADAR2. *Neuroscience* 95: 869–879
- Pennacchio LA, Bouley DM, Higgins KM, Scott MP, Noebels JL, Myers RM (1998) Progressive ataxia, myoclonic epilepsy and cerebellar apoptosis in cystatin B-deficient mice. *Nature Genet* 20: 251–258
- Pennacchio LA, Lehesjoki AE, Stone NE, Willour VL, Virtaneva K, Miao J, D'Amato E, Ramirez L, Faham M, Koskiniemi M, Warrington JA, Norio R, de la Chapelle A, Cox DR, Myers RM (1996) Mutations in the gene encoding cystatin B in progressive myoclonus epilepsy (EPM1). *Science* 271: 1731–1734
- Pucharcos C, Fuentes JJ, Casas C, de la Luna S, Alcantara S, Arbones ML, Soriano E, Estivill X, Pritchard M (1999) Alu-splice cloning of human Intersectin (ITSN), a putative multivalent binding protein expressed in proliferating and differentiating neurons and overexpressed in Down syndrome. *Eur J Hum Genet* 7: 704–712
- Ramjaun AR, McPherson PS (1996) Tissue-specific alternative splicing generates two synaptotagmin isoforms with differential membrane binding properties. *J Biol Chem* 271: 24856–24861
- Rouslin W (1991) Regulation of the mitochondrial ATPase in situ in cardiac muscle: role of the inhibitor subunit. *J Bioenerg Biomembr* 23: 873–888
- Sakisaka T, Itoh T, Miura K, Takenawa T (1997) Phosphatidylinositol 4,5-bisphosphate phosphatase regulates the rearrangement of actin filaments. *Mol Cell Biol* 17: 3841–3849
- Scott HS, Kudoh J, Wattenhofer M, Shibuya K, Berry A, Chrast R, Guipponi M, Wang J, Kawasaki K, Asakawa S, Minoshima S, Younus F, Mehdi SQ, Radhakrishna U, Papasavvas MP, Gehrig C, Rossier C, Korostishevsky M, Gal A, Shimizu N, Bonne-Tamir B, Antonarakis SE (2001) Insertion of beta-satellite repeats identifies a transmembrane protease causing both congenital and childhood onset autosomal recessive deafness. *Nat Genet* 27: 59–63
- Scott HS, Kyriakou DS, Peterson P, Heino N, Tahtinen M, Krohn K, Chen H, Rossier C, Lalioti MD, Antonarakis SE (1998) Characterization of a novel gene, C21orf2, on human chromosome 21q22.3 and its exclusion as the APECED gene by mutation analysis. *Genomics* 47: 64–70
- Slemmon JR, Morgan JI, Fullerton SM, Danho W, Hilbush BS, Wengenack TM (1996) Camstatins are peptide antagonists of calmodulin based upon a conserved structural motif in PEP-19, neurogranin, and neuromodulin. *J Biol Chem* 271: 15911–15917
- Slemmon JR, Hughes CM, Campbell GA, Flood DG (1994) Increased levels of hemoglobin-derived and other peptides in Alzheimer's disease cerebellum. *J Neurosci* 14: 2225–2235
- Stenmark H (2000) Membrane traffic: cycling lipids. *Curr Biol* 10: 57–59
- Tanoue A, Koshimizu TA, Tsuchiya M, Ishii K, Osawa M, Saeki M, Tsujimoto G (2002) Two novel transcripts for human endothelin B receptor produced by RNA editing/alternative splicing from a single gene. *J Biol Chem* 271 (in press)
- Turk V, Bode W (1991) The cystatins: protein inhibitors of cysteine proteinases. *FEBS Lett* 285: 213–219
- Utal AK, Stopka AL, Roy M, Coleman PD (1998) PEP-19 immunohistochemistry defines the basal ganglia and associated structures in the adult human brain, and is dramatically reduced in Huntington's disease. *Neuroscience* 86: 1055–1063
- Walker JE, Collinson IR (1994) The role of the stalk in the coupling mechanism of F1F0-ATPases. *FEBS Lett* 346: 39–43
- Veske A, Oehlmann R, Younus F, Mohyuddin A, Muller-Myhsok B, Mehdi SQ, Gal A (1996) Autosomal recessive non-syndromic deafness locus (DFNB8) maps on chromosome 21q22 in a large consanguineous kindred from Pakistan. *Hum Mol Genet* 5: 165–168
- Weitzdoerfer R, Fountoulakis M, Lubec G (2002) Reduction of actin-related protein complex 2/3 in fetal Down syndrome brain. *Biochem Biophys Res Commun* 293: 836–841
- Zheng J, Ramirez VD (1999) Rapid inhibition of rat brain mitochondrial proton F0F1-ATPase activity by estrogens: comparison with Na, K-ATPase of porcine cortex. *Eur J Pharmacol* 368: 95–102
- Ziai MR, Sangameswaran L, Hempstead JL, Danho W, Morgan JI (1988) An immunochemical analysis of the distribution of a brain-specific polypeptide, PEP-19. *J Neurochem* 51: 1771–1776
- Ziai R, Pan YC, Hulmes JD, Sangameswaran L, Morgan JI (1986) Isolation, sequence, and developmental profile of a brain-specific polypeptide, PEP-19. *Proc Natl Acad Sci USA* 83: 8420–8423

Authors' address: Prof. Dr. Gert Lubec, CChem, FRSC (UK) Department of Pediatrics, University of Vienna, Waehringer Guertel 18, A-1090 Vienna, Austria,
Fax: +43-1-40400-3194, E-mail: gert.lubec@akh-wien.ac.at