RESEARCH NOTE

Expression of *LDOC1* mRNA in leucocytes of patients with Down's syndrome

MICHELE SALEMI[1†], CONCETTA BARONE[2†], CARMELO ROMANO[1], FEDERICO RIDOLFO[1], ROBERTO SALLUZZO[1], FRANCESCO SCILLATO[1], CATALDO SCAVUZZO[1], FILIPPO CARACI[3], ALDO E. CALOGERO[4], CORRADO ROMANO[2] and PAOLO BOSCO[1*]

[1]*Laboratory of Cytogenetics and* [2]*Unit of Pediatrics and Medical Genetics, Oasi Institute for Research on Mental Retardation and Brain Aging, Troina, Italy*
[3]*Department of Pharmaceutical Sciences, University of Catania, Catania, Italy*
[4]*Section of Endocrinology, Andrology and Internal Medicine, Department of Internal Medicine and Systemic Diseases, University of Catania, Catania, Italy*

[Salemi M., Barone C., Romano C., Ridolfo F., Salluzzo R., Scillato F., Scavuzzo C., Caraci F., Calogero A. E., Romano C. and Bosco P. 2012 Expression of *LDOC1* mRNA in leucocytes of patients with Down's syndrome. *J. Genet.* **91**, 95–98]

Introduction

Down's syndrome (DS), or trisomy 21, results from an extra chromosome 21 which is due to failure of normal chromosomal segregation during meiosis. Individuals with trisomy 21 can develop premature ageing and some traits of Alzheimer disease at an earlier age than subjects without trisomy 21 (Wisniewski *et al.* 1985). These individuals were thought to experience a neurodegenerative process because of the presence of an extra copy of the amyloid precursor protein (APP) gene located on chromosome 21, but this hypothesis has not been entirely confirmed by the literature (Elsayed and Elsayed 2009; Arriagada *et al.* 2010). Several studies have amply demonstrated that genes related to apoptosis play a crucial role in neurodegenerative diseases, indeed apoptotic pathways appear to play a causal role in neurodegenerative processes and in cancer proliferation (Engidawork and Lubec 2001; Gulesserian *et al.* 2001; Seidl *et al.* 2001; Yoo *et al.* 2001; Engidawork and Lubec 2001; Fromage and Anglade 2002; Lubec and Engidawork 2002). The cancer process is favoured when the apoptotic surveillance is, for any reason (Hu and Kavanagh 2003), decreased; conversely, when the apoptotic process is some what encouraged, neurodegenerative processes, such as those related to Alzheimer disease, will be prevailing (Elmore 2007).

The leucine zipper, downregulated in cancer 1 (*LDOC1*) gene, was mapped on chromosome X at q27 and consists of only one exon (OMIM 300402). The Xq27 region is characterized by a high density of large segmental duplications (SDs) (Sharp *et al.* 2005). Because of the different orientation of SDs, their recombinational interactions may result in deletion, duplications, and inversion of the *LDOC1*-containing genomic region. Chromosomal rearrangements do not occur random, but result from a predisposition due to the existence of complex genomic architecture that may create instability in the genome (Shaw and Lupski 2004). Recently, Lawson and Zhang (2009) examined the genes that are located on the X-chromosomes of human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), mouse (*Mus musculus*) and rat (*Rattus norvegicus*) and identified gene families, including *LDOC1*, that show evidence of gene conversion.

LDOC1 gene codes for a nuclear protein, the 146 amino acid LDOC1 protein has a calculated molecular mass of approximately 17 kDa and contains a leucine zipper-like motif in its N-terminal region and a proline-rich region that shares marked similarity to an SH3-binding domain (Nagasaki *et al.* 1999). Northern blot analysis detected ubiquitous expression of *LDOC1* in normal tissues, with high expression in brain and thyroid and low expression in placenta, liver, and leucocytes (Nagasaki *et al.* 1999). The wide expression of *LDOC1* mRNA in normal tissues and the absence of *LDOC1* in most pancreatic and gastric cancer cell lines indicate that downregulation of *LDOC1* may have an important role in the development and/or progression of some cancers (Nagasaki *et al.* 2003). *LDOC1* is a known regulator of nuclear factor kappa B (NF-kappaB) that can affect the phorbol 12-myristate 13-acetate (PMA) or tumour necrosis factor-α (TNF-α) mediated pathway to apoptosis through inhibition of NF-kappaB (Nagasaki *et al.* 2003). Further, a

*For correspondence. E-mail: pbosco@oasi.en.it.
†These authors contributed equally to this work.

Keywords. Down's syndrome; *LDOC1* gene; mRNA; qRT-PCR expression.

transcription factor, myeloid zinc finger gene 1 (MZF-1), was revealed to interact with *LDOC1* and enhance the activity of *LDOC1* favouring apoptosis (Inoue *et al.* 2005). Finally, the expression of WAVE3 (Wiskott-Aldrich syndrome protein family, member 3), induces the translocation of LDOC1 from the nucleus to the cytoplasm, resulting in the inhibition of *LDOC1*-induced apoptosis, thus, it is possible to envisage that the *LDOC1* function is negatively regulated by *WAVE3* (Mizutani *et al.* 2001).

Considering these evidences, the aim of this study was to evaluate the possible differential expression of *LDOC1* mRNA in leucocytes of peripheral blood of DS subjects compared with the normal population.

Materials and methods

A total of 40 subjects were enrolled for this study at the Unit of Pediatrics and Medical Genetics of the IRCCS Oasi Institute, Troina (Italy), a specialized centre for patients with intellective disability mainly from Sicily. They included: 20 DS patients (9 males and 11 females) with a mean age of 39.05 ± 10.15 (range 20–55 years) and 20 normal subjects (9 males and 11 females) with a mean age of 38.95 ± 10.86 (range 19–55 years). The 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. The RNA quality and quantity were checked by spectrophotometry.

To avoid any genomic DNA contamination during qRT-PCR, a brief incubation of the samples at 42°C with a specific Wipeout buffer (QuantiTect Reverse Transcription kit, Qiagen Sciences, Germantown, USA) was carried out. Retrotranscription of 600 ng of total RNA from each sample was then performed in a final volume of 20 μL and generated cDNA was used as a template for real-time quantitative PCR analysis using gene expression products. For each sample real-time PCR reactions were carried out in duplicate using 2.5 μL of cDNA and QuantiTect Probe PCR Master Mix kit (Qiagen Sciences, Germantown, USA) in a total volume of 50 μL. *LDOC1* and *GAPDH* assays were obtained from Applied Biosystems (Carlsbad, CA, USA). The thermal cycling conditions consisted of one cycle for 2 min at 50°C, one cycle of 15 min at 95°C and 40 cycles for 15 s at 94°C followed by 1 min at 60°C. Real-time analysis was performed on Light Cycler 480 (Roche Diagnostics, Mannheim, Germany). The amplified transcripts were quantified using the comparative CT method (Livak and Schmittgen 2001), relative quantification data analysis was played using the comparative $\Delta\Delta$Ct method included in the software version 1.5 supplied with the LightCycler 480 and finally chi-square test statistical analysis was performed with Graph Pad Prism 5 software (GraphPad software, La Jolla, USA). *LDOC1* gene expression level was normalized to *GAPDH* level (Mauro *et al.* 2011) and Target Mean Cp definition was used to indicate the mean normalized cycle threshold.

Results and discussion

In our case–control study, *LDOC1* gene expression was increased in 15 (75%) DS samples compared to normal age and sex-matched subjects (table 1). A statistically significant result was obtained using the chi-square test ($P = 0.0016$). Six of the samples (DS3, DS8, DS10, DS12, DS13, and DS15 in table 1) have shown an expression at least doubled in comparison to normal samples. Further, 2 of 15 samples of DS subjects (DS1, and DS18 in table 1) have shown a *LDOC1* expression greater than 1.5 compared to the corresponding normal sample.

LDOC1 has been shown to inhibit the activation of NF-kappaB through ligand-induced stimulation by TNF-α or PMA in a dose-dependent manner and viability studies demonstrated that TNF-α or PMA-induced antiproliferative effects were significantly enhanced by stable transfection of cells with *LDOC1* (Nagasaki *et al.* 2003). These observations suggested that LDOC1 was a novel regulator of NF-kappaB that can affect the PMA or TNF-alpha-mediated pathway to apoptosis through inhibition of NF-kappaB (Nagasaki *et al.* 2003). Further, overexpression of *LDOC1* causes externalization of the cell membrane phosphatidylserine, which is characteristic for early-phase apoptotic events, and reduced cell viability in some human cell lines (Inoue *et al.* 2005). Mizutani *et al.* (2001) have shown that ectopically expressed LDOC1 is localized in the nucleus and induces apoptosis, accompanied by an increase in the tumour protein p53 (p53) protein level, but not in p53 transcription, suggesting that LDOC1 inhibits the degradation of p53. Lynn *et al.* (2009), in a study of expression array on to male young-onset hypertension, have suggested that innate immune response and cell-proliferation regulation may play important downstream roles in development of hypertension and specifically that *LDOC1* gene plays a key role in the regulatory mechanisms related to apoptosis in hypertension.

Our preliminary data suggest a potential role for *LDOC1* gene as a marker of the apoptotic mechanisms working in DS. However, the data obtained from our experiments need to be validated confirming the link between overexpression of *LDOC1* and activation of the apoptotic pathways both in early ageing and neurodegenerative processes in DS. Currently, an evaluation of the expression of the *LDOC1* gene in other tissues and in cell cultures of fibroblasts obtained from subjects with DS is in progress, to increase our knowledge and strengthen the evidence obtained.

Further experimental studies will be focussed on the comparison between *LDOC1* overexpression, activation of the caspase pathways and apoptotic cell death in a larger series of individuals affected by DS and/or Alzheimer's disease in order to confirm the potential role of LDOC1 as a er for apoptosis in DS and in neurodegenerative diseases.

Table 1. *LDOC1* gene expression in DS and normal controls.

Sample name	Age (years)	Sex	Target mean Cp (*LDOC1* gene)	Reference mean Cp (*GAPDH* gene)	Ratio normalized
N1	30	M	33.13	25.13	1.000
DS1	32	M	32.49	25.23	1.671
N2	19	F	28.09	20.96	1.000
DS2	20	F	28.33	20.92	0.824
N3	25	F	28.28	20.60	1.000
DS3	27	F	27.55	20.98	2.116
N4	55	F	29.08	22.84	1.000
DS3	55	F	29.93	23.80	1.083
N5	41	M	28.03	21.71	1.000
DS5	39	M	28.48	22.21	1.039
N6	31	F	32.05	24.52	1.000
DS6	31	F	30.25	22.87	1.103
N7	41	M	27.83	20.79	1.000
DS7	42	M	28.11	17.92	0.447
N8	32	M	34.05	41.61	1.000
DS8	35	M	26.43	35.06	2.122
N9	52	M	26.36	33.80	1.000
DS9	51	M	27.20	33.51	0.458
N10	48	F	27.73	37.47	1.000
DS10	50	F	30.30	41.14	2.153
N11	25	F	29.00	21.19	1.000
DS11	27	F	26.67	19.12	1.115
N12	43	F	26.86	19.71	1.000
DS12	45	F	27.87	23.60	4.773
N13	44	F	29.26	31.32	1.000
DS13	45	F	28.88	32.19	2.397
N14	44	F	29.26	31.32	1.000
DS14	44	F	29.10	31.32	1.113
N15	44	F	29.26	31.32	1.000
DS15	47	F	32.74	35.95	2.123
N16	52	M	37.80	30.23	1.000
DS16	47	M	40.87	33.75	1.372
N17	41	M	25.60	30.20	1.000
DS17	39	M	24.93	29.33	0.880
N18	19	F	33.13	25.13	1.000
DS18	20	F	32.49	25.23	1.670
N19	43	M	26.96	32.65	1.000
D19	41	M	27.16	30.90	0.300
N20	47	M	31.64	30.98	1.000
D20	47	M	31.06	30.72	1.252

Cp, crossing points; N, normal subject; DS, Down's syndrome patient.

References

Arriagada C., Bustamante M., Atwater I., Rojas E., Caviedes R. and Caviedes P. 2010 Apoptosis is directly related to intracellular amyloid accumulation in a cell line derived from the cerebral cortex of a trisomy 16 mouse, an animal model of Down syndrome. *Neurosci. Lett.* **470**, 81–85.

Elmore S. 2007 Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* **35**, 495–516.

Elsayed S. M. and Elsayed G. M. 2009 Phenotype of apoptotic lymphocytes in children with Down syndrome. *Immun. Ageing* **6**, 6–12.

Engidawork E. and Lubec G. 2001 Protein expression in Down syndrome brain. *Amino Acids.* **21**, 331–361.

Fromage B. and Anglade P. 2002 The aging of Down's syndrome subjects. *Encephale* **28**, 212–226.

Gulesserian T., Engidawork E., Yoo B. C., Cairns N. and Lubec G. 2001 Alteration of caspases and other apoptosis regulatory proteins in Down syndrome. *J. Neural Transm. Suppl.* **61**, 163–179.

Hu W. and Kavanagh J. J. 2003 Anticancer therapy targeting the apoptotic pathway. *Lancet Oncol.* **4**, 721–729.

Inoue M., Takahashi K., Niide O., Shibata M., Fukuzawa M. and Ra C. 2005 LDOC1, a novel MZF-1-interacting protein, induces apoptosis. *FEBS Lett.* **579**, 604–608.

Lawson M. J. and Zhang L. 2009 Sexy gene conversions: locating gene conversions on the X-chromosome. *Nucleic Acids Res.* **37**, 4570–4589.

Livak K. J. and Schmittgen T. D. 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* **4**, 402–408.

Lubec G. and Engidawork E. 2002 The brain in Down syndrome (TRISOMY 21). *J. Neurol.* **249**, 1347–1356.

Lynn K. S., Li L. L., Lin Y. J., Wang C. H., Sheng S. H., Lin J. H. *et al.* 2009 A neural network model for constructing endophenotypes of common complex diseases: an application to

male young-onset hypertension microarray data. *Bioinformatics* **25**, 981–988.

Mauro M. O., Sartori D., Oliveira R. J., Ishii P. L., Mantovani M. S. and Ribeiro L. R. 2011 Activity of selenium on cell proliferation, cytotoxicity, and apoptosis and on the expression of CASP9, BCL-XL and APC in intestinal adenocarcinoma cells. *Mutat. Res.* **715**, 7–12.

Mizutani K., Koike D., Suetsugu S. and Takenawa T. 2001 WAVE3 functions as a negative regulator of LDOC1. *J. Biochem.* **138**, 639–646.

Nagasaki K., Manabe T., Hanzawa H., Maass N., Tsukada T. and Yamaguchi K. 1999 Identification of a novel gene, LDOC1, down-regulated in cancer cell lines. *Cancer Lett.* **140**, 227–234.

Nagasaki K., Schem C., von Kaisenberg C., Biallek M., Rösel F., Jonat W. *et al.* 2003 Leucine-zipper protein, LDOC1, inhibits NF-kappaB activation and sensitizes pancreatic cancer cells to apoptosis. *Int. J. Cancer* **105**, 454–458.

Seidl R., Bidmon B., Bajo M., Yoo P. C., Cairns N., La Casse E. C. *et al.* 2001 Evidence for apoptosis in the fetal Down syndrome brain. *J. Child Neurol.* **16**, 438–442.

Sharp A. J., Locke D. P., McGrath S. D., Cheng Z., Bailey J. A., Vallente R. U. *et al.* 2005 Segmental duplications and copy-number variation in the human genome. *Am. J. Hum. Genet.* **77**, 78–88.

Shaw C. J. and Lupski J. R. 2004 Implications of human genome architecture for rearrangement-based disorders: the genomic basis of disease. *Hum. Mol. Genet.* **1**, 57–64.

Wisniewski K. E., Dalton A. J., McLachlan C., Wen G. Y. and Wisniewski H. M. 1985 Alzheimer's disease in Down's syndrome: clinicopathologic studies. *Neurology* **35**, 957–961.

Yoo B. C., Vlkolinsky R., Engidawork E., Cairns N., Fountoulakis M. and Lubec G. 2001 Differential expression of molecular chaperones in brain of patients with Down syndrome. *Electrophoresis* **22**, 233–241.

Received 7 February 2011, in revised form 24 October 2011; accepted 29 November 2011
Published on the Web: 13 March 2012