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LETTER TO THE EDITOR

NF-kB1 gene expression in Down syndrome patients

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Dear Sir,

Down syndrome (DS) is the most common human genetic disorder. In DS 95 % are free trisomies and the remainder are mosaics or have translocations [1, 2].

The phenotype of Down syndrome is characterized by distinctive physical appearance, hypotonia, immunological defects, endocardial, hematological and endocrinal alterations, behavioral and cognitive deficits and mental retardation [1, 2]. Numerous studies have documented the presence in DS subjects of senile plaques and neurofibrillary tangles, hallmarks of Alzheimer's disease [1–3]. Apoptotic pathways in neurodegenerative processes are determinant [1–3]; conversely, when the apoptotic process is some what encouraged, neurodegenerative processes, such as those related to Alzheimer disease, will be prevailing, therefore, becomes important to evaluate the apoptotic processes in DS [5].

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Nuclear factor-kB1 (NF-kB1) regulates the transcription of many genes involved in immune response, cell adhesion, differentiation, proliferation, angiogenesis and apoptosis [4]. The gene encodes p105 and p50 proteins, p105 is a non-DNA-binding cytoplasmic molecule and p50 is a DNA-binding protein corresponding to the N-terminus of p105 (OMIM, 164011).

Increased *NF-kB1* activation is involved in inflammatory response [4]. *NF-kB1* signaling plays an essential role in tumor development and aggressiveness by enhancing angiogenesis, proliferation, anti-apoptosis, and repressing immune response [5]. The aim of this study was to evaluate the possible differential expression of NF-kB1 mRNA using qRT-PCR in fibroblasts obtained from oral biopsy of periodontal gingival tissue of DS subjects compared with normal subjects.

The DS cases and the controls were recruited after family and/or personal informed consent at the IRCCS Oasi of Troina (Italy), a specialized centre for mental retardation and brain aging studies. Human fibroblasts were obtained from oral biopsy of periodontal gingival tissue in eight normal subjects (4 male and 4 female; age range 32–45 years) and eight DS subjects (4 male and 4 female; age range 28–45 years). Human fibroblasts were isolated from explants of human gingiva and cultured in DMEM (Dulbecco Modified Eagles Medium) in 5 % CO_2 humidified atmosphere supplemented with fetal bovine serum (FBS), 2 mM glutamine and 100 units/ml of streptomycin and penicillin.

A suspension containing 5×10^6 fibroblasts/ml in culture medium was treated with the RNeasy Mini Handbook (QIAGEN Sciences, Germantown, PA, USA), following the manufacturer's protocol.

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Table 1 *NF-KB1* mRNA expression in fibroblasts of Down syndrome subjects and normal controls

Sample name	Age (years)	Sex	Target mean Cp (*NF-KB1gene*)	Reference mean Cp (*GAPDH* gene)	Ratio normalized
C1	45	M	31.82	31.90	1.000
DS1	45	M	27.92	26.97	0.475
C2	32	F	31.81	31.92	1.000
DS2	31	F	31.44	29.51	0.242
C3	32	M	30.68	31.91	1.000
DS3	28	M	27.32	27.72	0.557
C4	35	F	31.80	31.92	1.000
DS4	31	F	29.11	27.72	0.354
C5	36	F	40.00	36.57	1.000
DS5	34	F	31.87	27.73	0.792
C6	45	M	21.89	19.07	1.000
DS6	45	M	26.09	23.05	0.850
C7	33	F	27.39	23.25	1.000
DS7	33	F	31.12	26.86	0.933
C8	32	M	39.15	23.25	1.000
DS8	28	M	44.00	26.87	0.214

Cp crossing points, *C* normal subject, *DS* Down syndrome patients

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, PA, USA) was carried out. Retro-transcription 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. 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 LightCycler 480 (Roche Diagnostics; Mannheim, Germany). The amplified transcripts were quantified using the comparative CT method and relative quantification analysis data were played using the comparative ΔΔCt method included in the Software Version 1.5 supplied with the LightCycler 480. *NF-kB1* gene expression level was normalized to *GAPDH* level. QRT-PCR analysis shows, in all eight subjects with DS, a down-expression of *NF-kB1* compared to fibroblasts from normal subjects (Table 1). The minor expression of *NF-kB1* in fibroblasts of DS subjects compared to fibroblasts of normal donors may relate to the association of this gene to the same pathway of expression that in this case favors the activation of the pro-apoptotic mechanisms in DS [5]. In fact, inhibition of *NF-kB1* has been linked directly to apoptosis, inappropriate immune cell development and delayed cell growth [5]. In conclusion, our preliminary data suggest a potential role for gene *NF-kB1* as a marker of the apoptotic mechanisms acting in Down syndrome. However, the data obtained from our experiments need to be validated on a more extended cohort.

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