Cellular & Molecular Biology
Cell. Mol. Biol. 2015; 61 (3): 6-7
Published online June 2, 2015 (http://www.cellmolbiol.com)
Received on April 22, 2015, Accepted on May 23, 2015.
doi : 10.14715/cmb/2015.61.3.2

Letter to Editor: Cytochrome b gene expression in down syndrome subjects

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Down syndrome (DS) is caused by an extra copy of chromosome 21. The phenotype of DS is complex and many aspects are variable, but intellectual disability (ID) and the early development of Alzheimer's disease (AD) are common to all individuals (1). The incidence of DS is approximately one in 730 live births in the United States and approximately one in 1,000 within Europe. (2,3). Some authors suggested that mitochondrial DNA (mtDNA) alterations have a role in the pathogenesis of DS as premature ageing, diabetes and development of AD (4,5,6,7). It is established that the mtDNA alterations increase with age in different cells, such as oocytes. This could affect the non disjunction of chromosome 21 in oocytes, and may lead to a decreased mitochondrial function with a consequent increase of the neurotoxic mechanisms (6,7).

Cytochrome b (*MTCYB*) is the only mtDNA encoded subunit of respiratory complex III. Complex III is the second enzyme in the electron transport chain of mitochondrial oxidative phosphorylation and is located within the mitochondrial inner membrane. It catalyzes the transfer of electrons from ubiquinol (reduced Coenzyme Q10) to cytochrome c and utilizes the energy to translate protons from inside the mitochondrial inner membrane to outside (OMIM *516020).

The aim of this study was to evaluate *MTCYB* mRNA expression in fibroblasts of DS subjects versus fibroblasts of normal control subjects. Human gingival fibroblasts were cultured in Dulbecco Modified Eagles Medium (DMEM) in 5% CO_2 humidified atmosphere supplemented with fetal bovine serum (FBS), 2 mM glutamine and 100 units/ml of streptomycin and penicillin. Total mRNA of fibroblasts was obtained by a suspension of fibroblasts ($5 \cdot 10^6$ fibroblasts/mL). The study was performed on a total of 16 subjects. Eight DS patients (5 males and 3 females; age range 25-57 years) and 8 normal subjects (5 males and 3 females; age range 22-55 years). The DS cases and the controls were recruited at the IRCCS Associazione Oasi Maria SS at Troina (Italy), a specialized centre for mental retardation and brain aging studies. This study was approved by the Ethical Committee of the Research Institute "IRCCS Associazione Oasi Maria SS.", Troina (EN), Italy. The Ethical Committee, chaired by Prof. Salvino Leone, approved the project on June 17, 2013 (Prot. N. CE2013/06/17).

Total mRNA from fibroblasts was obtained as described by Salemi et al. (2012) (9). RNA quantity and purity were confirmed by spectrophotometry and agarose gel electrophoresis. 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) was carried out.

Retro-transcription of 650 ng of total RNA from each sample was then performed in a final volume of 30µl and generated cDNA was used as a template for real-time quantitative PCR analysis using gene expression products. For each sample qRT-PCR reactions were carried out in duplicate using 4 µl of cDNA and QuantiTect Probe PCR Master Mix Kit (QIAGEN Sciences, Germantown, PA) in a total volume of 25 µl.

QRT-PCR experiments were performed using the Light Cycler 480 (Roche Diagnostics; Mannheim, Germany). The target *MTCYB* gene (ID TaqMan Assay Hs02596867-s1) and the reference gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (ID TaqMan Assay GAPDH Hs99999905-ml) 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 42 cycles for 15 s at 94 °C followed by 1 min at 60 °C. The amplified transcripts were quantified using the comparative CT method and relative quantification analysis data were played using the comparative ΔΔCt method. *MTCYB* gene expression level was normalized to *GAPDH* level and Target Mean Cp definition was used to indicate the mean normalized cycle threshold. In fibroblasts, *MTCYB* gene expression was increased in 6 of the 8 DS samples compared with age and sex-matched control subjects (Table 1): 3 of them (Table 1) showed an expression at least doubled in comparison with normal control samples. Two of the eight cases have a lower expression of *MTCYB* gene compared with the corresponding controls, this could mean that other alterations associated to the expression of mitochondrial genes influence the phenotype of DS. Alterations of expression in mtDNA may result in an increased generation of free radicals and decreased ATP levels. The latter could affect not only synaptonemal complex, chromosome segregation and cellular division, but also recombination leading to aneuploidy (10,11). MtDNA's impaired expression may lead to an altered mitochondrial function with a consequent greater vulnerability

Table 1. Cytochrome b gene expression in fibroblasts of DS and normal controls.

Sample Name	Age (yars)	Sex	Target Mean Cp (Cytocrome b)	Reference Mean Cp (GAPDH gene)	Ratio Normalized
N1	30	M	32.01	36.24	1.000
DS1	32	M	33.22	35.60	0.277
N5	41	M	36.66	40.08	1.000
DS5	39	M	31.00	36.34	3.779
N2	19	F	35.83	39.26	1.000
DS2	20	F	37.21	42.30	3.183
N3	25	M	35.57	39.28	1.000
DS3	27	M	33.44	38.93	3.444
N4	55	F	33.59	40.04	1.000
DS3	55	F	35.29	40.52	0.431
N6	31	F	29.46	31.93	1.000
DS6	31	F	30.68	30.60	1.368
N7	41	M	39.45	31.93	1.000
DS7	42	M	40.27	36.30	1.801
N8	32	M	39.35	32.20	1.000
DS8	35	M	41.15	34.53	1.444

Cp: crossing points; N: normal subject; DS: Down syndrome patient

to neurotoxic insults (12). Alterations of mitochondrial function and enhanced oxidative stress influence the expression of chromosome 21 genes (12), and act directly on protein expression of various mitochondrial complexes (12,13). Some authors concluded that mitochondrial dysfunction in the DS brain may contribute to the pathogenesis of AD: oxidative stress may interfere with beta amyloid precursor protein (AβPP) processing (14). Another study showed mitochondrial dysfunction in AD fibroblasts: ATP levels were significantly reduced in these fibroblasts (15). These data on expression of *MTCYC* mtDNA in DS individuals are consistent with this concept. Furthermore, these data confirm the essential role of mitochondrial function in DS, not only in the onset of AD, but also for the general phenotype of DS patients. Our results are preliminary , we hope in the near future to be able to increase the cases to confirm the data obtained.

Acknowledgements

This study was supported by funds obtained from the Italian Ministry of Health (Ricerca Corrente).

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