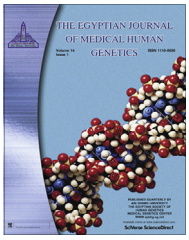
The Egyptian Journal of Medical Human Genetics (2013) **14**, 307–310

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

Assessment of immune function in Down syndrome patients

Ekram Abdel-Salam [a,*], **Iman Abdel-Meguid** [a], **Soheir Korraa** [b]

[a] *Department of Pediatrics, Genetic Unit, Faculty of Medicine, Cairo University, 11 Falaki Square, Cairo 11111, Egypt*
[b] *Department of Radiation Health, National Center for Radiation Research and Technology, Egypt*

Received 9 April 2013; accepted 23 May 2013
Available online 21 June 2013

KEYWORDS

Down syndrome;
Cystathionine beta synthase (CBS);
H_2S;
Calcineurin activity (CAN);
Tumor necrosis factor-α (TNF-α);
Interlukin-2 (IL-2)

Abstract In Down syndrome (DS), trisomy 21 leads to overexpression of gene coding for specific enzymes. This overexpression translates directly into biochemical aberrations that affect multiple interacting metabolic pathways which culminates in cellular dysfunction and contributes to the unique pathogenesis of DS. The aim of this study is to evaluate parameters of immune response in terms of cytokines [tumor necrosis factor-α (TNF-α) and interlukin-2 (IL-2)] together with the quantitative expression of cystathionine beta synthase (CBS), whose transsulfuration pathway generates cysteine and hydrogen sulfide (H_2S). H_2S is known to boost host defense at physiological concentrations and to display cytotoxic activity at higher concentrations. Calcineurin activity (CAN) was also measured as its dysregulation has been shown to cause immune suppression. Subjects were 60 DS patients vs. 30 age and socioeconomic matching healthy controls. In their blood, the cytokines: TNF-α and IL-2, together with CBS and its by product H_2S as well as CAN activity, were measured. Results showed that CBSmRNA relative expression (0.56 ± .06 vs. 0.32 ± .02), plasma H_2S (72 ± 8.5 vs. 50.8 ± 4.1) and TNF-α (8.11 ± .01 vs. 3.6 ± 0.9) were significantly higher among DS patients compared to controls, while CAN (0.27 ± 0.1 vs. 0.45 ± 0.2 units), was significantly decreased in blood of DS patients compared to controls. IL-2 (36.4 ± 2.6 vs. 37.4 ± 0.9) showed no significant difference between DS patients and controls. Accordingly it can be concluded that excessive synthesis of multiple gene products derived from overexpression of the genes present on chromosome 21 may be the cause for decreased immunity in DS patients compared to controls.

1. Introduction

The molecular basis for DS is the presence of an extra copy of chromosome 21, referred to as trisomy 21 (Ts21). Chromosome 21 contains 303 genes [1]. The excessive synthesis of multiple gene products derived from over expression of the genes present on chromosome 21 is thought to underlie both the dysmorphic features and the pathogenesis of the neurological, immunologic, endocrine, and biochemical abnormalities that are characteristic of DS [2]. Recent studies have reported that young people with Down syndrome suffer from low-grade systemic inflammation [3].

The Down syndrome critical region (DSCR1) protein, now renamed RCAN1 (''Regulator of Calcineurin'') [4], is over-expressed in the brain of Down syndrome fetuses and

* Corresponding author. Tel.: +20 1006868206; fax: +20 37833449.
E-mail address: ekab@link.net (E. Abdel-Salam).
Peer review under responsibility of Ain Shams University.

ELSEVIER Production and hosting by Elsevier

1110-8630 © 2013 Production and hosting by Elsevier B.V. on behalf of Ain Shams University.
http://dx.doi.org/10.1016/j.ejmhg.2013.05.003

interacts physically and functionally with CAN. It was found that the 1.5-fold increase in dosage of RCAN1 destabilizes a regulatory circuit, responsible for many of the features of Down syndrome [5]. It is suggested that RCAN1 overexpression, inhibits CAN [6]. Recently, it has been reported that the gene RCAN1 contained within the DSCR, acts to prevent the nuclear occupancy of transcription factors responsible for CAN expression. CAN plays a critical role in regulating T cell development and activation [7]. In activated T lymphocytes, CAN dephosphorylates the nuclear factor of activated T cells and promotes its translocation into the nucleus and the up-regulation of early T-lymphocyte genes such as the Th1-cytokine interleukin IL-2 [8,9].

Situated on DSCR1 is the gene controlling CBS, an enzyme that catalyzes the first of two steps in the transsulfuration pathway that converts homocysteine into cysteine. A 157% increase in CBS enzyme activity has been previously documented in individuals with DS and has been associated with reduced levels of homocysteine and production of H_2S [10]. Physiological functions of endogenous H_2S have been found to play an important role as a signal molecule in regulating cell survival [11]. It appears paradoxical that, on one side, H_2S acts as a physiological intercellular messenger to stimulate cell growth, and on the other hand H_2S at higher concentration is known to be cytotoxic. Proliferating T cells depend on their endogenous capacity to generate H_2S, and this capacity is enhanced by T cell activation [12]. In addition to this autocrine role, H_2S may have a paracrine function in T cell activation at normal concentration. This idea is supported by the report of increased H_2S output from activated antigen presenting cells [13].

TNF-α is an early and potent pro-inflammatory cytokine that stimulates the inflammatory response and is involved in the apoptosis pathway which is injurious [14]. IL-2 is also a key factor in multiple processes involved in the survival of T lymphocytes and in the regulation of T cell responses. It plays an important role in the regulation of anergy because the anergic $CD4^+$ T cells are characterized by their inability to proliferate and express IL-2 following TCR-specific stimulation in the presence of adequate costimulation [15,16]. It has been proposed that anergy results from TCR activation in the absence of IL-2 production [17,18].

Accordingly, CAN activity and CBS mRNA expression and H_2S together with the cytokines: TNF-α and IL-2, were measured in blood of DS patients and compared to those of controls.

2. Patients and methods

Patients were 60 Down syndrome cases (33 males and 27 females). Age ranged from 2 to 15 years (mean 9.3 ± 4.1 years). Controls were 30 matched healthy normal children (18 males and 12 females), their age ranged from 2 to 14 years (mean 9.1 ± 4.2 years). DS patients were selected from cases already diagnosed by chromosomal karyotyping in the genetics unit, pediatric department, Cairo University. Blood was drawn after an informed consent of the parents in accordance with the current revision of the 1975 Helsinki declaration.

3. Measurement of plasma H_2S concentration

Plasma sample (0.1 ml) was added to a test tube containing 0.5 ml of 1% zinc acetate and 2.5 ml of distilled water, then 0.5 ml of 20 mmol/L N,N-dimethyl-p-phenylenediaminedihydrochloride in 7.2 mol/L HCl and 0.4 ml of 30 mmol/L FeCl3 in 1.2 mol/L HCl were also added to the same test tube for 20 min of incubation at room temperature. The protein in the plasma was removed by adding 1 ml of 10% trichloroacetic acid to the solution and centrifuging it. The optical absorbance of the resulting solution at 670 nm was measured with a spectrometer [19].

4. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for CBSmRNA

Phytohemagglutinin stimulated lymphocytes were used for RNA extraction since unstimulated leukocytes contain no detectable CBS activity [20]. Total RNA was extracted from lymphocytes using QIAGEN RNA extraction kit (QIAGEN Inc., USA). The RNA samples were reverse transcribed using a blend of Sensiscript and Omniscript reverse transcriptases; using QIAGEN One step RT-PCR kit (QIAGEN Inc. USA). The cDNA product was amplified using 2.5 units of HotStarTaq DNA polymerase, 1 mmol $MgCl_2$/l, and 0.1 umol of the CBS primer 5′-GGG CAC ACC ATC GAG ATC CTC-3′and 5′-AGA GCC TGC CCA GCG TGT C-3′ [21]. β-actin primers were: 5′-GTG GGG CGC CCC AGG CAC CA-3′; and 5′-CTC CTT AAT GTC ACG CAC GAT TTC-3′. Five microlitre of RT reaction of each cDNA was processed for PCR. Ten microlitre from each PCR reaction product was separated on a 2% agarose gel then stained with ethidium bromide. The appearance of specific bands at 205 and 540 bp for CBS and b-actin, respectively, using a 50 bp molecular weight marker, was evaluated under ultraviolet light and photographed.

5. ELISA IL-2

The amounts of IL-2 in circulating lymphocytes were determined by a sandwich enzyme linked immunosorbent assay (ELISA) Predicta IL-2 Kit [22].

TNF-α plasma analysis of the cytokine TNF-α was performed using ELISA R & D kit [23].

5.1. Calcineurin activity

The assay was carried out using the AK-804 BIOMOL GREEN Quantizyme Assay System (Biomol) Bueno et al. [24].

6. Results

Results showed that calcineurin activity (0.27 ± 0.1 vs. 0.45 ± 0.2 units) was significantly decreased among DS patients compared to controls, while CBSmRNA expression (0.56 ± .06 vs. 0.32 ± .02) (Fig. 1) and H_2S (72 ± 8.5 vs. 50.8 ± 4.1) were significantly higher among DS patients compared to controls (Table 1). TNF-α (8.11 ± .01 vs. 3.6 ± 0.9) was significantly higher in blood of DS patients compared to controls and IL-2 (36.4 ± 2.6 vs. 37.4 ± 0.9) showed no

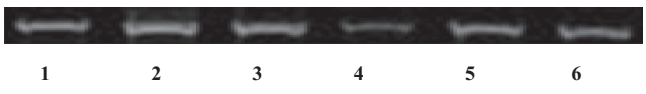


Figure 1 Cystathionine beta synthase mRNA in blood of DS patients (lanes 1–4) compared to controls (lanes 1–3).

Table 1 Calcineurin and cystathionine beta synthase in blood of DS patients.

	Down syndrome	Controls	*p*
Calcineurin activity	0.27 ± 0.1 units	0.45 ± 0.2 units	<0.001
CBSmRNA relative expression	0.56 ± .06	0.32 ± .02	<0.001
H_2S μmol/L	72 ± 8.5	50.8 ± 4.1	<0.001

Table 2 Cytokines in blood of DS patients.

	Down syndrome	Controls	*p*
TNF	8.11 ± .01	3.6 ± 0.9	<0.001
IL-2	36.4 ± 2.6	37.4 ± 0.9	<0.05

significant differences between DS patients and controls (36.4 ± 2.6 vs. 37.4 ± 0.9) as shown in Table 2.

7. Discussion

The excessive synthesis of multiple gene products derived from overexpression of the genes present on chromosome 21 is thought to underlie both the dysmorphic features and the pathogenesis of the neurological, immunologic, endocrinal, and biochemical abnormalities that are characteristic of DS. The successful management of the clinical problems and unique pharmacological sensitivities of these children is a major medical challenge and depends on understanding of the unique metabolic imbalance induced by overexpression of the constitutively expressed genes on chromosome 21 [2].

Results of the present study revealed a significant decrease in calcineurin activity among DS patients compared to controls. The reason for this decrease is attributed to a gene located on the region DSCR1 inhibiting calcineurin [2]. It has been demonstrated that RCAN1 protein is overexpressed in the brain of Down syndrome fetuses, and interacts physically and functionally with calcineurin [2]. Overexpression of RCAN1 and RCAN inhibits calcineurin-dependent gene transcription through the inhibition of its transcription factor (NAFT). CAN plays a critical role in regulating T cell development and activation [7].

There was a significant increase in **CBSmRNA** among DS patients compared to controls. A 157% increase in CBS enzyme activity has been previously documented in individuals with DS and has been associated with reduced levels of homocysteine and increased level of H2S [25,26]. An increased level of H_2S is known to cause memory and motor function deficits and is cytoxic to T-cells [27,11].

In the present study there was a significant increase in cytokine TNF in DS patients compared to controls. This result although contradictory to the study of Cetiner et al., who showed decreased levels in TNF among DS children [28], is in agreement with previous studies that demonstrated the same as our results [29,30]. Also *in vitro* incubated DS blood stimulated with 2.5×10 [3] TCID50/ml influenza A virus showed a significant rise in TNF compared with control children. It has been assumed that the production of higher levels of pro-inflammatory cytokines (TNF) may be responsible for a more severe clinical course of viral disease in these children [31], since TNF is involved in the apoptosis pathway [14].

Previous studies are controversial regarding IL-2 production by stimulated lymphocytes in DS patients. While a study showed decreased levels of IL-2 among stimulated lymphocytes [32], another study showed increased levels of IL-2 among DS patients compared to controls [33]. Results of the present study showed no significant differences in IL-2 in DS patients compared to controls. Other studies showed that the decreased immunity among DS patients is due to decreased lymphocyte subsets and that the primary immune defect in DS is in part a depressed number and function of helper T cells which stimulate Il-2 [34].

Accordingly it can be concluded that excessive synthesis of multiple gene products derived from overexpression of the genes present on chromosome 21 may lead to decreased immunity in DS patients compared to controls.

References

[1] Sinet P, Théophile D, Rahmani Z, Chettouh Z, Blouin J, Prieur M, et al. Mapping of the Down syndrome phenotype on chromosome 21 at the molecular level. Biomed Pharmacother 1994;48(5–6):247–52.

[2] Korenberg J, Chen X, Schipper R, Sun Z, Gonsky R, Gerwehr S, et al. Down syndrome phenotypes: the consequences of chromosomal imbalance. Proc Natl Acad Sci USA 1994;91:4997–5001.

[3] Ordonez F, Fornieles-Gonzalez G, Rosety M, Rosety I, Diaz A, Rosety-Rodriguez M. Anti-Inflammatory Effect of Exercise Via Reduced Leptin Levels in Obese Women with Down Syndrome. Int J Sport NutrExercMetab. 2013;23(3):239–44.

[4] Davies K, Ermak G, Rothermel B, Pritchard M, Heitman J, Ahn J, et al. Renaming the DSCR1/Adapt78 gene family as RCAN: regulators of calcineurin. FASEB J 1998;21(12):3023–8.

[5] Crostin N, Serra A, Rigo A, Viglino P. Dosage effect of SOD-A gene in 21-trisomic cells. Hum Genet 1976;31:197–202.

[6] Silveira H, Sommer C, Soares-Costa A, Henrique-Silva F. Calcineurin inhibitory protein overexpressed in Down's syndrome interacts with the product of a ubiquitously expressed transcript. Braz J Med Biol Res 2004;37(6):785–9.

[7] Doetschman T, Sholl A, Chen Dd, Gard C, Hildeman D, Bommireddy R. Divergent effects of calcineurin Aβ on regulatory and conventional T-cell homeostasis. Clin Immunol 2011;138(3):321–30.

[8] McCaffrey P, Luo C, Kerppola T, Jain J, Badalian T, Ho A, et al. Isolation of the cyclosporin-sensitive T cell transcription factor NFATp. Science 1993;262:750–4.

[9] Batiuk T, Halloran P. The downstream consequences of calcineurin inhibition. Transplant Proc 1997;29:1239–40.

[10] Singh S, Padovani D, Leslie R, Chiku T, Banerjee R. Relativecontributions of cystathioninebeta-synthase and gamma-cystathionase to H2S biogenesis via alternative trans-sulfuration reactions. J Biol Chem 2009;284(33):22457–66.

[11] Yang G. Hydrogen sulfide in cell survival: a double-edged sword. Expert Rev Clin Pharmacol 2011;4(1):33–47.

[12] Miller T, Wang E, Gould S, Stein E, Kaur S, Lim L, et al. Hydrogen sulfide is an endogenous potentiator of T cell activation. J Biol Chem 2012;287(6):4211–21.

[13] Zhu X, Liu S, Wang S, Ni X. Glucocorticoids suppress cystathionine γ-lyase expression and H_2S production in lipopolysaccharide-treated macrophages. Cell Mol Life Sci 2010;67:1119–32.

[14] Kuru S, Inukai A, Kato T, Liang Y, Kimura S, Sobue G. Expression of tumor necrosis factor-alpha in regenerating muscle fibers in inflammatory and non-inflammatory myopathies. Acta Neuropathol (Berlin) 2003;105:217–24.

[15] Davis L, Lipsky P. Tolerance induction of human $CD4^+$ T cells: markedly enhanced sensitivity of memory vs. naive T cells to peripheral anergy. Cell Immunol 1993;146:351–61.

[16] Maier C, Greene M. Biochemical features of anergic T cells. Immunol Res 1998;17:133–40.
[17] Schwartz R. A cell culture model for T lymphocyte clonal anergy. Science 1990;248:1349–56.
[18] Paiardini M, Galati B, Cervasi G, Cannavo G, Galluzzi L, Montroni M, et al. Interleukin-2 administration corrects the cell cycle perturbation of lymphocytes from human immunodeficiency virus-infected individuals. J Virol 2001;75:10843–55.
[19] Abe K, Kimura H. The possible role of hydrogen sulphide as an endogenous neuromodulator. J Neurosci 1996;16:1066–71.
[20] Taigen T, De Windt L, Lim H, Molkentin J. Targeted inhibition of calcineurin prevents agonist-induced cardiomyocyte hypertrophy. Proc Natl Acad Sci USA 2000;97:1196–201.
[21] You X, Xu C, Lu J, Zhu X, Ga L, Cui X, et al. Expression of cystathionine β-synthase and cystathionine γ-lyase in human pregnant myometrium and their roles in the control of uterine contractility. PLoS One 2011;6(8):e23788.
[22] Nakata B, Chung K, Kato Y, Yamashita Y, Inui A, Arimoto Y, et al. Serum soluble interleukin-2 receptor level as a prognostic indicator in gastric cancer. Br J Cancer 1998;77(11):1820–4.
[23] Saito K, Kobayashi K, Sasaki M, Araake H, Kida T, Yagihashi A, et al. Detection of human serum tumor necrosis factor-in healthy donors, using a highly sensitive immuno-PCR assay. Clin Chem 1999;45:665–9.
[24] Chadefaux B, Ceballos I, Hamet M, Poissonnier M, Kamon P, Allard D. Is absence of atheroma in Down syndrome due to decreased homocysteine levels? Lancet 1988;2:741–6.
[25] Chadefaux B, Rethore M, Raoul O, Ceballos I, Poissonnier M, Gilgenkranz S, et al. Cystathionine beta synthase: gene dosage effect in trisomy 21. Biochem Biophys Res Commun 1985;128(1985):40–4.
[26] Kamoun P. H2S, a new neuromodulator. Med Sci (Paris) 2004;20(6–7):697–700.
[27] Chen J, Jhee K, Kruger W. Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H2S biogenesis via alternative trans-sulfuration reactions. J Biol Chem 2009;279(50):52082–6.
[28] Cetiner S, Demirhan O, Inal T, Tastemir D, Sertdemir Y. Analysis of peripheral blood T-cell subsets, natural killer cells and serum levels of cytokines in children with Down syndrome. Int J Immunogenet 2010;37(4):233–7.
[29] Shimada A, Hayashi Y, Ogasawara M, Park MJ, Katoh M, Minakami H, et al. Pro-inflammatory cytokinemia is frequently found in Down syndrome patients with hematological disorders. Leuk Res 2007;31(9):1199–203.
[30] Nateghi Rostami M, Douraghi M, Miramin Mohammadi A, Nikmanesh B. Altered serum pro-inflammatory cytokines in children with Down's syndrome. Eur Cytokine Netw 2012;23(2):64–7.
[31] Broers CJ, Gemke RJ, Weijerman ME, van der Sluijs KF, Van Furth AM. Increased pro-inflammatory cytokine production in Down syndrome children upon stimulation with live influenza A virus. J Clin Immunol 2012;32(2):323–9.
[32] Goldstein J, Campbell B, Gartler S. Cystathionine synthase activity in human lymphocytes: induction by phytohemagglutinin. J Clin Invest 1972;51:1034–7.
[33] Gerez L, Madar L, Arad G, Sharav T, Reshef A, Ketzinel M, et al. Aberrant regulation of interleukin-2 but not of interferon-gamma gene expression in Down syndrome (trisomy 21). Clin Immunol Immunopathol 1991;58(2):251–66.
[34] Bertotto A, Arcangeli, Crupis S, Marinelli I, Gerrli R, Vaccaro R. T cell response to anti-CD3 antibody in Down's syndrome. Arch Dis Child 1987;62:1148–51.