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A peculiar VNTR in the *cystathionine β-synthase* gene is a risk factor for Down Syndrome

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Abstract

In the present study, we analysed a 31bp variable number of tandem repeats (VNTR) of the cystathionine ß-synthase (*CBS*) gene in 427 subjects: 127 patients with Down syndrome (DS) and in 60 of their mothers; 172 age-and sex-matched controls and in 68 of their mothers. A significant statistical difference in the distribution of the 21 repeat allele was found comparing mothers of subjects with DS versus mothers of children without DS ($\chi 2$= 4.166; P = 0.0413; Table 2). Since *CBS* 21 repeats allele carriers show a decrease of *CBS* enzyme activity possibly leading to lower intracellular glutathione concentration, these results could be explained by a higher not disjunction probability of chromosome 21 in oocytes, due to poor antioxidative protection against reactive oxygen species (ROS) toxic activity.

Key words: Cystathionine ß-synthase, glutathione, Down syndrome, VNTR, oocytes.

Introduction

Down's syndrome (DS), or trisomy 21, is the most frequent genetic cause of intellectual disability (ID). It results from the gene expression of the extra chromosome 21, which occurs due to the failure of normal chromosomal segregation during meiosis. A genetic defect of the homocysteine metabolism may be associated with increased chromosomal instability (1) and in genetic disorders such neural tube defect and DS (2). Homocysteine may catabolized by the trans-sulfuration pathway where cystathionine β-synthase (*CBS*) irreversibly condensates homocysteine in cystathionine (3,4). Untreated CBS deficiency causes abnormalities in tissues and cells (5,6). *In vivo*, *CBS* removes homocysteine from the methionine cycle, and commits it to the trans-sulfuration pathway of cysteine and glutathione (GSH) synthesis (3,7). Glutathione is the most abundant antioxidant molecule in mammalian cells (3,4,7). Deficits in GSH have been implicated in aging and a host of diseases including DS (8,9). GSH has been implicated in maintaining the meiotic spindle morphology of the oocytes (10,11).

A 31 bp variable number of tandem repeats (VNTR) is present between exon13 and intron13 of the *CBS* gene. It consists of 17, 18, 19 or 21 repeat units that specifies four different alleles (12). Molecular defects in genes encoding enzymes involved in homocysteine metabolism may account for mild hyperhomocysteinaemia (12). In the present study, we analysed this 31 bp VNTR of the *CBS* gene in DS patients, mothers of subjects with DS and controls, to assess the contribution of genetic variability on the occurrence of non-disjunction as proposed by Eppig JJ (1996) and Krisher RL, Bavister BD (1998) (13,14).

Materials and methods

A total of 427 subjects were enrolled in this study at the IRCCS Oasi Institute, Troina (Italy). In this specialized center receiving mainly patients from the whole Sicily. They included 127 DS patients (80 males and 47 females, age range 33-45 years), 60 mothers of subjects with DS (age range 36-67 years), 172 normal subjects (97 males and 75 females; age range 29-50 years), and 68 age-matched control mothers (age range 33-67 years). Karyotype analysis revealed free 21 trisomy for all DS patients. 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). DNA was isolated from a lymphocyte-enriched fraction of whole blood (15). Primers for PCR were: forward primer 5'FAM-TGCAGCCGTCAGACCAAG-3' and reverse primer 5'-TTAAGTCCCCAAAACACGG-3' (12). Capillary electrophoresis was performed using POP7 gel and ABI PRISM 3130 Genetic Analyser (Applied Biosystems, USA). Fragments' sizes and peaks' areas were determined by GeneScan Analysis 3.1.2 software (Applied Biosystems, USA) (16,17,18). The difference in distribution of allele frequencies were assessed with the chi square test. A *P* value lower than 0.05 was considered to indicate statistical significance.

Results

The allele frequencies of the 31 bp VNTR of the *CBS* gene found in 127 DS subjects and 172 controls are shown in Table 1. No difference was observed comparing alleles 17, 18, 19 and 21 in DS and controls subjects. In the mothers of subjects with DS versus mothers of children without DS no difference was observed com-

Table 1. Allele frequency of Cystathionine ß synthase, VNTR allele 17, 18, 19 and 21 in Down Syndrome subjects and controls.

	n DS (total n= 127) frequence;	n controls (n= 172) frequence ;	χ2 test (P-value)
CBS 31bp VNTR			* χ2 2.705; P 0.4394
allele 17	28 0.07 %	34 0,10 %	
allele 18	292 0,77 %	246 0,72 %	
allele 19	43 0,12 %	45 0,13 %	
allele 21	18 0,04 %	19 0,05 %	

n DS: Down Syndrome; *: Continuity-corrected χ2 test with three degree of freedom.

Table2. Allele frequency of Cystathionine ß synthase, VNTR allele 17, 18, 19 and 21 in Down Syndrome mothers and controls mothers.

	n DS m (total n= 60) ;	c. mothers (n= 68) frequence;	χ2 test (P-value)
CBS 31bp VNTR			* χ2 5.050; P 0.1682
allele 17	7 0.06 %	13 0.10 %	
allele 18	86 0.72 %	101 0.74 %	
allele 19	11 0.09 %	14 0.10 %	
allele 21	16 0.13 %	8 0.06 %	** χ2 4.166; P 0.0413

DS m: Down Syndrome mothers; c. mothers: controls mothers; *: Continuity-corrected χ2 test with three degree of freedom; **: Continuity-corrected χ2 test with one degree of freedom (allele 17, 18 and 19 vs 21).

paring alleles 17, 18, 19 and 21; however, a significant statistical difference was found between the 21 repeat allele vs. all the others ($\chi 2$= 4.166; P = 0.0413; Table 2).

Discussion

The 31 bp VNTR of the *CBS* gene is a genetic determinant and is associated with variations of levels of the homocysteine (19). In a mathematical model study of glutathione metabolism, CBS enzyme activity decreased in *CBS* 21 repeats allele, and leads to a reduction of GSH and an increase in oxidative stress (8). Glutathione is involved in many pathways that are essential for normal intracellular homeostasis (8,12). GSH is a natural antioxidant present in both sex gametes, its level varies widely, and has been implicated in the fertilization process and embryo development (13). In mature oocytes, GSH plays an important role in pro-nucleus formation after fertilization (1,14,20). In fact, GSH is active in oocyte function, including meiotic spindle morphology (9). Cumulus cells surrounding oocytes are involved in the process of GSH synthesis (5,6,20). The present study found a significant statistical difference in mothers of subjects with DS versus mothers of children without DS, in the distribution of the 21 repeats allele of 31 bp VNTR *CBS* gene, specifically difference was found between the 21 repeat allele vs. all the others alleles ($\chi 2$= 4.166; P = 0.0413; Table 2). In conclusion, CBS enzyme activity, decreased in 21 repeat allele among the mothers of subjects with DS could lead to non-disjunction of chromosome 21 in oocyte, because GSH may be implicated in maintaining the meiotic spindle morphology of the oocyte.

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