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CYSTATHIONINE BETA SYNTHASE : GENE DOSAGE EFFECT IN TRISOMY 21

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The enzymatic activity of cystathionine beta synthase has been studied in fibroblasts of nine patients with regular trisomy 21. An excess of CBS activity was found in trisomy 21 with a trisomy 21/normal ratio equal to 1.66. A 1.04 ratio was found in 21q21 ⟶ 21 p ter monosomy ; a 1.04 and 0.99 ratio was found in two 21 qter ⟶ 21q22.3 monosomies ; a 1.14 ratio in 21 qter ⟶ 21q22 monosomy ; a 0.89 ratio in a 21q21 ⟶ 21 pter trisomy ; an excess of CBS activity was found in a 21q22.1 ⟶ 21q21 trisomy with a 1.57 ratio. These results show a gene dosage effect in human fibroblasts trisomic for chromosome 21 and suggest the assignment of human CBS locus between 21q22.1 and 21q21.

Cystathionine beta synthase (CBS ; EC 4.2.1.21) is the enzyme in the mammalian transsulfuration pathway which catalyses the condensation of homocysteine and serine to cystathionine, this being an intermediate step in the conversion of methionine to cysteine (1). The CBS deficiency is the cause of the most frequent disease of sulfur amino acid metabolism, the homocystinuria, and then this enzyme has been extensively studied. Recently Skovby et al.(2) reported the results of Chinese hamster/human somatic cell hybrid studies which indicated that the locus for CBS maps to chromosome 21. To evaluate further the assignment of the gene of CBS to chromosome 21 and to determine whether a gene dosage effect exists, we have studied the expression of this enzyme in trisomic 21 and normal fibroblast strains.

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MATERIALS AND METHODS

Fibroblast strains : fibroblast cultures were prepared from direct skin biopsies of nine patients with regular trisomy 21, ten normal subjects, two patients with partial trisomy 21 and four patients with partial monosomy 21. Cells were grown at 37°C in plastic flasks (Falcon) in RPM 1640 medium (Boehringer) supplemented with N-2 hydroxymethylpiperazine N'-2 ethane sulfonic acid (Hepes) : 4 g/l ; bicarbonate : 2g/l ; 10 % newborn calf serum (Flow laboratories) and penicillin + streptomycin (100 units and 0,2 mg/ml respectively). Care was taken to assure that all cultures were harvested at the same stage of confluence. After reaching confluence the cultures were fed consecutively for three days and harvested on the fourth day. Cells were collected in phosphate buffer saline at 4°C and centrifuged at 300 g for 5 min. Two additional washes were conducted and, after the last centrifugation, the cells were subjected to three cycles of freezing-thawing. Cell debris were removed by centrifugation at 1 500 g for 5 min. The extracts were stored frozen at - 70°C until the time of assay.

Enzymatic assay : CBS was assayed according to the method of Fleisher et al. (3). Cystathionine formed was measured directly on an automatic amino acid analyser (TSM 1 Technicon) according to the method of Castets et al. (4). Protein concentrations were determined according to the method of Lowry et al. (5). Enzymatic activity is expressed as nmol of cystathionine formed per mg of protein per hour at 37°C.

Case Reports : Case 1 : At the time of the examination this slightly mentally retarded proposita was 17 years old. She had trisomy 21q21 $\longrightarrow$ 21pter and trisomy 15q26.2 due to malsegregation of a maternal balanced t (15 ; 21) (q26.2 ; q21) previously reported by Raoul et al. (6). Case 2 : This patient was a 16-year-old-boy who was found to have 21q22.1 $\longrightarrow$ 21q21 trisomy (46, XY, dup dir (21) (q21 ; q22.1). Both parents had normal karyotypes (7). Case 3 : The propositus was a 15-year-old-boy with severe mental retardation. His unbalanced karyotype, trisomy 15 pter $\longrightarrow$ 15q13 and monosomy 21q21 $\longrightarrow$ 21pter was the result of a maternal balanced t (15;21) (q13;q22.1) (8). Case 4 : This girl, who died when she was three months old, was found to have an unbalanced karyotype with trisomy 9 pter $\longrightarrow$ 9p13 and monosomy 21qter $\longrightarrow$ 21q22.3 resulting from a paternal balanced t (9 ; 21) (p 13 ; q 22.2) (unpublished observation). Case 5 : The patient was a 7-month-old-boy with partial monosomy 21qter $\longrightarrow$ 21q22.3 and trisomy 16 q 22.3 resulting from a paternal balanced t (16 ; 21) (q 22.2 : q 22.2). (9). Case 6 : The patient was a 21 month-old-girl with monosomy 21qter $\longrightarrow$ 21q22 and trisomy 15qter $\longrightarrow$ 15q22 resulting from a maternal balanced t (15 ; 21) (q22 ; q22) (10).

RESULTS

In cultured fibroblasts the CBS activity in the trisomic cells was significantly greater than in normal cells with respectively 23.54 $\pm$ 3,12 nmol/mg/h and 14.2 $\pm$ 2,03

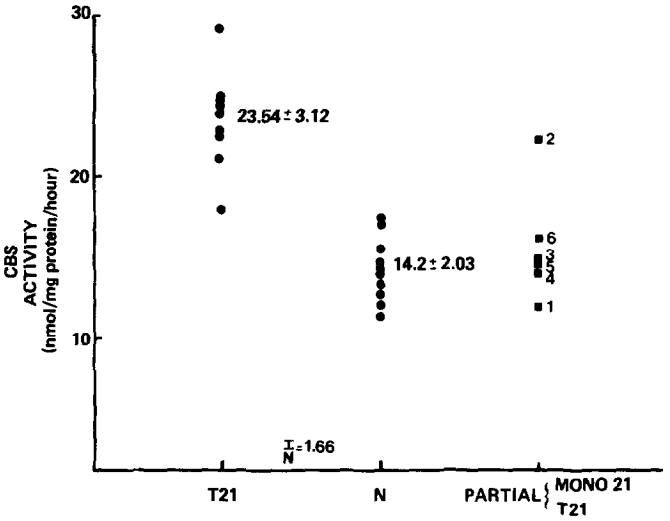

Fig. 1. Activity of CBS in extracts of trisomy 21 and normal human fibroblasts. The mean ratio of trisomy 21 to normal (T/N) activities was 1.66. Each value is the mean of three determinations.

nmol/mg/h (mean $\pm$ SD) ($p < 0,001$). The mean ratio of trisomy 21 to normal (T/N) was 1,66.(Fig. 1). A 1.04 ratio was found in 21q21 $\longrightarrow$ 21pter monosomy ; a 1.04 and 0.99 ratio was found in two 21qter $\longrightarrow$ 21q22.3 monosomies ; a 1.14 ratio in 21qter $\longrightarrow$ 21q22 monosomy ; a 0.89 ratio in a 21q21 $\longrightarrow$ 21pter trisomy ; an excess of CBS activity was found in a 21q22.1 $\longrightarrow$ 21q21 trisomy with a 1.57 ratio (Table 1) (Fig. 2).

Table I : Patients and cystathionine beta synthase assay

Case	Sex	Age at assay	Parent carrier	Trisomy 21 or Monosomy 21	Breakpoint of Translocation	Assay of cystathionine synthase (nmol/mg/hour)
1	F	17 y	Mother	Trisomy 21q21 $\longrightarrow$ 21pter	(15;21) (q26.2;q21)	12.1
2	M	16 y	De novo	Trisomy 21q22.1 $\longrightarrow$ 21q21	dup dir 21 (q21:q22.1)	22.34
3	M	15 y	Mother	Monosomy 21q21 $\longrightarrow$ 21pter	(15;21) (q13;q22.1)	14.78
4	F	3 m	Father	Monosomy 21qter $\longrightarrow$ 21q22.3	(9;21) (p13;q22.2)	14.12
5	M	7 m	Father	Monosomy 21qter $\longrightarrow$ 21q22.3	(16;21) (q22.2;q22.2)	14.72
6	F	20 m	Mother	Monosomy 21qter $\longrightarrow$ 21q22	(15;21) (q22;q22)	16.23

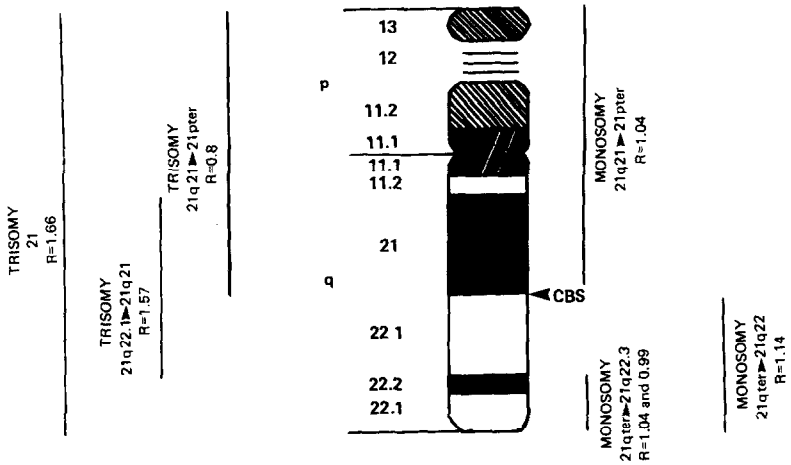

Fig. 2. Localization of the CBS locus on chromosome 21.

DISCUSSION

The 1.66-fold increase in mean CBS activity in fibroblasts obtained from individuals with trisomy 21 is quite close to the expected gene dosage effect of 1.5.Therefore, this finding, which add yet another enzyme to the list of enzymes for which the expected gene dosage effect is demonstrable also serve to confirm the mapping of CBS to chromosome 21. The enzymatic activity of CBS in several cases of partial monosomies and trisomies 21 suggest the assignment of human CBS locus between 21q22.1 and 21q21. Therefore the gene of CBS, as cytosol superoxide dismutase (11), antiviral receptor protein (12), phosphoribosylglycinamide synthetase (13), is in the region of the chromosome 21 which need to be trisomic to cause Down's syndrome. But nothing is known regarding the biochemical basis of the disease. Because of published suggestions (14) that a significant mechanism operative in trisomy 21 might be the existence of abnormal methylation mechanisms the chances are relatively high that CBS does play a role in the pathology of Down's syndrome. It may be that this increase in CBS synthesis causes overproduction of cystathionine and cysteine and perturbs specific methylation mechanisms. This possibility is now under investigation in our laboratory.

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