International Archives of Medical Research
Volume 2, No. 2, pp.54-56, 2011.

ORIGINAL RESEARCH PAPER

Cystathionine synthase T833C/844ins68 Polymorphism: A family –based study on down syndromes children

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Abstract

Cystathionine β-synthase (CBS) mediates conversion of homocysteine to cystathionine and deficiency in enzyme activity may be lead to hyperhomocysteinemia/homocystinuria, which are often associated with Down Syndrome (DS). A large number of polymorphisms have been reported in the CBS gene, some of which

impair its activity and among these, a T833C polymorphism in cis with a 68 bp insertion at 844 in the exon 8 is found to be associated with mild hyperhomocysteinemia in different ethnic groups. Our aim in the present study is to investigate the association between T833C/844ins68 polymorphism and DS.

Methods: Fifty-seven DS cases parents (mothers) were recruited after psychometric evaluation. Peripheral blood was collected after obtaining informed written consent. The T833C/844ins68 polymorphism was investigated by PCR amplification of genomic DNA

Results: After PCR Analysis 15 samples were found to have +/- genotype while 42 samples were found to have -/- genotype for CBS 844ins68 polymorphisms.

Conclusion: This is the first molecular genetic study of CBS gene dealing with T833C/844ins68 double mutation in DS subjects in our region. The next step is to extended number of cases and to use more controls for T833C/844INS68 polymorphism.

Keywords: Down Syndrome, CBS gene, T833C/844ins68 polymorphism

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Introduction

Down syndrome (DS) is caused by trisomy of either the entire amount, or a critical portion of chromosome 21. Birth prevalence increases with maternal age from 0.6 in 1,000 live births at 20 years up to 11 in 1,000 live births at 40 years.1 In 95% of cases, DS is caused by a meiotic error occurring at meiosis I or II, mainly of maternal origin (1).Cystathionine -synthase (CBS) catalyzes the condensation of serine and homocysteine to form cystathionine and abnormality in CBS activity is manifested in two major clinical conditions, viz. Hyperhomocysteinemia and homocystinuria. Insufficiency in CBS activity may lead to hyperhomocysteinemia , which is considered to be an independent risk factor for arteriosclerosis. The human *CBS* gene, located at 21q22.3 , is known to have a large number of mutations, the majority of which are missense in nature . Three different nonsense mutants, as well as some insertion, deletion and splice variants have also been identified, some of which are polymorphic in nature(2). 844ins68 is a frequent polymorphism of the cystathionine-synthase gene (CBS) that consists of a 68-bp insertion duplicating the 3_ splice site of intron 7 and the 5-end of exon 8. The presence of two identical 3_ splice sites spaced by 68 bp should lead to either a selection of the proximal site or to at least two alternatively spliced CBS mRNA variants (3). Our aim in the present study is to investigate the association between T833C/844ins68 polymorphism and DS.

Materials and Methods

The study was based on 34 recruited mothers who had children affected by DS below 35 years of age. Genomic DNA was extracted from peripheral leukocytes employing standard methods.12 DNA analysis was carried out by PCR amplification of a DNA fragment containing exon 8 of the CBS gene as previously described.6 The PCR products were eletrophoresed on a 2% agarose gel and photographed under UV light after ethidium bromide staining. The resulting fragments were 252-bp in the presence and 184-bp in the absence of the 844ins68 CBS insertion. CBS 844ins68: Primers for amplification were: 5'-CTGCCTTGAGCCCTGAAGCC- 3', 5'-CTGGACTCGACCTACCGTCCT-3'. PCR conditions were: denaturation 94°C (1 minute), annealing 60°C (1 minute), elongation 72°C (1 minute) for a total of 39 cycles. When present, the 844ins68 insertion caused a +68bp shifted band of the PCR product (242 bp instead of 174 bp).

Results and Discussion

Down syndrome is the main genetic cause of mental retardation and a major public health concern. Despite the high incidence and social impact of DS, the available knowledge of its etiology and pathogenesis is incomplete.The risk of a Down syndrome (DS) pregnancy is a function of maternal age, and after age 35 years it increases substantially with increasing maternal age. However, several women aged less than 35 years at conception have a child with DS, suggesting a predisposition to early chromosome malsegregation events in such women Folate is an important nutrient which is required for both DNA synthesis and methylation. Several studies performed on human cell cultures, in vivo studies in humans and studies involving animal models have demonstrated that folate depletion from the media, or inadequate folate dietary intake, result in DNA hypomethylation, chromosome breakage, increased frequency of micronuclei (MN) and aneuploidy. In conclusion, results here indicate that MDS are more prone than controls to chromosome damage and malsegregation events, and suggest that, among four of the major folate and homocysteine metabolizing gene polymorphisms previously associated with the risk of a DS pregnancy in Italy Scala et al., 2006(1).

This study; after PCR Analysis 15 samples were found to have +/- genotype while 42 samples were found to have -/- genotype for CBS 844ins68 polymorphisms. These results provide further evidence for a major role of gene-gene interactions in the assessment of genetic susceptibility to DS. It can be hypothesized that blood folates and homocysteine levels influence the impact of the genotype.This is the first molecular genetic study of *CBS* gene dealing with T833C/844ins68 double mutation in DS subjects in our region.

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