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Original Article

Maternal Betaine Homocysteine Methyltransferase Gene Polymorphism as a Risk Factor for Trisomy

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Disorder in re-methylation process of homocysteine to methionine due to mutation in betaine homocysteine methyltransferase enzyme (BHMT) coding gene, leads to decrease in S-adenosyl methionine (SAM) synthesis which takes part in DNA methylation as a methyl donor. As a result, it can promote hypo-methylation of DNA, chromosome instability, and chromosome missegregation, which in turn is one of the main risk factors in trisomy 21 occurrence. The aim of this study was to investigate the distribution of *BHMT* polymorphism among mothers of Down syndrome and normal children. Genomic DNA extracted from blood samples of 45 mothers with at least one child presenting Down syndrome, as test group, and 30 mothers without affected children, as control group. G> A Single nucleotide polymorphism rs3733890 was investigated by PCR-RFLP method. The frequency of A allele was 37% in test group and 20% in control group. The frequency of G allele was 63% and 80% in test and control groups, respectively. The abundance of homozygous GG genotype was higher in control group (P= 0.03; OR GG = 1 and OR AA, AG = 1.4). Higher frequency of A allele in mothers with children affected with Down syndrome compared to control group, indicates that there might be an involvement of *BHMT* gene polymorphism in chromosomal nondisjunction leading to trisomy 21 children birth.

Keywords: Down syndrome, betaine homocysteine methyltransferase (BHMT), RFLP

Autosomal trisomy 21 is the most common chromosomal disorder which is due to an additional copy of chromosome 21. It is the consequence of chromosomal nondisjunction. Its incidence is 9 in each 10,000 live birth and it is almost equal in different races (1). Its occurrence is also 1 in each 150 pregnancy and is responsible for 80% of failed pregnancies (2) Specific genes on

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chromosome 21 code for transcription factors which their over-production due to additional copy of chromosome leads to inhibition of some essential genes, taking part in formation of heart, muscle and neurons (3).

Although a long time has been passed from the discovery of chromosomal basis of DS, underlying cellular and molecular mechanisms of the chromosomal nondisjunction is not fully understood yet (2, 4, 5). However higher age of mothers is considered as a main risk factor, but many of the affected children are born from mothers under 30 years old (2, 4, 6). Low age mothers that give birth to DS children are supposed to have a genetic susceptibility for early chromosomal nondisjunction (7).

It has been reported that both genetic and environmental factors are important in etiology of DS (8). Nondisjunction of chromosomes is a multifactorial trait and a DS child may have been influenced by genetic factors, epigenetic factors, and environmental factors (7). Methylation is the most important epigenetic mechanism which has a key role in genomic stability and gene regulation (9). Disorder in DNA methylation increases the potential of chromosomal nondisjunction. Based on this hypothesis, hypo-methylation of centromere DNA leads to abnormalities in formation of kinetochore and microtubules which can prone the cells to chromosomal nondisjunction (2, 5). Epigenetic DNA methylation, histones methylation, and heterochromatinization processes occur coordinate-ly in order to stabilize DNA and chromosomes (6). Methylation is conducted by an enzyme family called DNA methyltransferases (DNMTs) in which S-adenosyl methionine is used as methyl group donor and DNA is used as substrate (10). Methionine is one of the four sulfur amino acids of the body. It is also the precursor of SAM synthesis which in turn acts as a biological methyl group donor to DNA and RNA during transmethylation reactions (4, 11). Since methionine is produced in remethylation cycle of homocysteine, insufficient remethylation processes (Figure 1) results in decrease of methionine and SAM production (9). Deficiency of methyl group donor leads to hypomethylation of DNA in centromere region which cause chromosomal instabilities such as deletion, translocation, and missegregation of chromosomes (6). During remethylation process of homocysteine, receiving methyl group can achieved by an ammonium compound called betaine or trimethylglyceride (TMG). Transfer of methyl group from betaine to homocysteine via betaine-homocysteine S-methyltransferase (BHMT), produces dimethylglycine (DMG) and methionine (11, 12). This enzyme is only expressed in liver and kidney and its expression is much fewer in other tissues. Choline is the source of betaine and BHMT enzyme substrate. Betaine is yielded from both choline catabolism and dietaries (13). Insufficient choline consumption and varied activity BHMT enzyme due to genetic polymorphisms can cause biological disorders in methylation process which consequently increases the risk of DS birth (14). Betaine can effectively help to decrease of homocysteine level of plasma (15).

BHMT gene in human is located on chromosomal location 5q14.1 (base pairs 78407604-78428113) (16). Transition (G742A) occurs at nucleotide 742 in exon 6 of the gene which leads to replacement of glutamine with arginine in structure of the enzyme. This enzyme catalysis rate is fewer compared to the wild type (11, 12). Related polymorphisms generate two separated alloenzymes which are fewer in wild type species. Therefore, betaine and homocysteine levels of mutant group and polymorph group are different significantly when compared to wild type. Low levels of the alloenzymes indicate the increased efficiency of

homocysteine remthylation. The polymorphism is considered as a risk factor in mothers for giving birth to a DS child (11, 17). It is also combined with coronary artery disease in fetus, spinal injuries, oral cleft, carcinoma and various types of cancer (15). When the destiny of homocysteine metabolites are changed, hyperhomocysteinemia appears which in turn have a critical rol in pathobiology. There are some evidences that indicate there are a correlation between homocysteine with increased levels of oxidative stress, DNA damages, and apoptosis development (18). Hyperhomocysteinemia is developed by either dietary factors or genetic factors including functional polymorphisms in key enzymes of homocysteine metabolism (19). It is reported that plasma homocysteine concentrations is higher in mothers with DS children compared to mothers with healthy children. There are also lower methionine levels in plasma of mothers with DS children (2, 4).

The above mentioned findings suggest that impaired homocysteine disorders may be due to genetic polymorphisms of metabolic enzymes which sensitize individuals to chromosomal damages and may thus be a risk factor for giving birth to DS children (11). Therefore, in the present study the role of rs3733890 has been analyzed as one of the maternal risk factors for giving birth to DS children in Mazandaran province population of Iran.

Materials & methods

Subjects

45 mothers with DS children (confirmed karyotypes) were considered as test group. There were also 30 mothers with healthy children as control group. These cases were introduced by gifted education organization, state welfare organization, and rehabilitation centers. Both groups were matched for age, education, financial status. Informed consent was obtained from all mothers.

Molecular analysis

Five ml fresh peripheral blood was collected from each participant in EDTA containing tubes. Genomic DNA was extracted from leukocytes using salting out standard method combined with protein kinase K. The quality of extracted DNA was controlled via 2% agarose gel electrophoresis. DNA samples were stored at -20 ºC for further use. In order to analysis BHMT gene polymorphism rs3733890, a 171 bp fragment was amplified by PCR method using forward 5'-TGCTGGTTTCTGG TGCATCCCTAA-3' and reverse 5'-AAGGGCTG ACTCATCAGGTGAGCT TTGAGT-3' primers (12). PCR reactions were performed in 25 µl total volume containing 1 µl DNA sample, 1.5 mM MgCl2, 200 µM dNTP, 0.2 mM each forward and reverse primers, 1 unit Taq polymerase enzyme and 1X buffer. PCR reactions started with primary denaturation at 95 ºC for 2 min, followed by 1 min at 95 ºC, 1 min at 62.5 ºC and 1 min at 72 ºC for 35 cycles, the final extension lasted 4 min at 72 ºC. After completion of PCR, the quality of amplicons was monitored using 2% agarose gel (Figure 1).

In the next step, 171 bp amplicons underwent enzymatic digestion using *HinfI* restriction enzyme in a final volume of 20 µl by overnight incubation at 37 ºC. Then restricted fragments were electrophoresed on 12% polyacrylamide gel and DNA bands were visualized after silver nitrate staining (Figure 2). Digestion of A allele generated

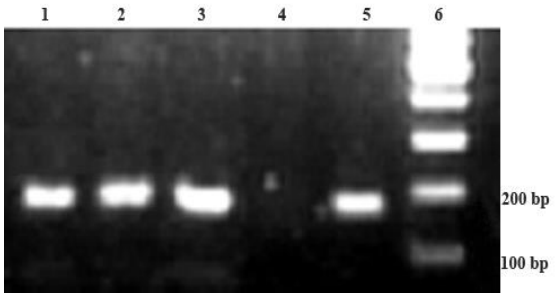

Figure 1. 171 bp fragment of PCR product of BHMT gene (lanes 1-3, 5) with NTC (lane 4) and 100bp DNA ladder (lane 6).

160 and 11 bp fragments while the G allele generated 141, 19 and 11 bp fragments.

Statistical analyzes

Data were analyzed by SPSS software (version 19) and using Chi square test (χ^2). $P < 0.05$ was considered as statistically significant.

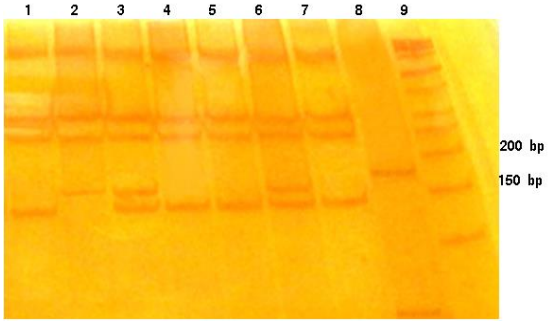

Figure 3. Analysis of rs3733890 genotypes by RFLP method. Lanes 1, 4, 5 and 7: GG homozygous (141 bp); lanes 3 and 6: heterozygous (160 & 141 bp); lanes 2: homozygous AA (160 bp); lane 8: Undigested PCR product (171 bp); lane 9: 50bp DNA ladder.

Results

The analysis of mothers' age shows that 75.5% of mothers with DS child were under 35 years old. Mothers under 25 years old were the most represented (Figure 3). Table 1 shows the rs3733890 allele and genotype frequencies of BHMT in two groups. The allelic frequency in test group was 63% and 37% for G and A alleles respectively and 80% and 20% respectively in control group showing a higher frequency of A allele in the test group (PValue> 0.05). Heterozygous AG genotype had the most frequency (51%) in test group, while homozygous GG genotype had the most frequency (63.3%) in control group. This genotypic distribution difference was statistically significant between the two groups (P Value = 0.03; OR GG = 1 and OR AA, AG = 1.4) Therefore it seems that the chance of giving birth to a DS child is dependent on mothers' genotype.

DS is one of the common chromosomal disorders worldwide with high complexity in phenotypic expression. Therfore, investigating the genetic basis predisposing to disease development and controlling the disease has a considerable

Discussion

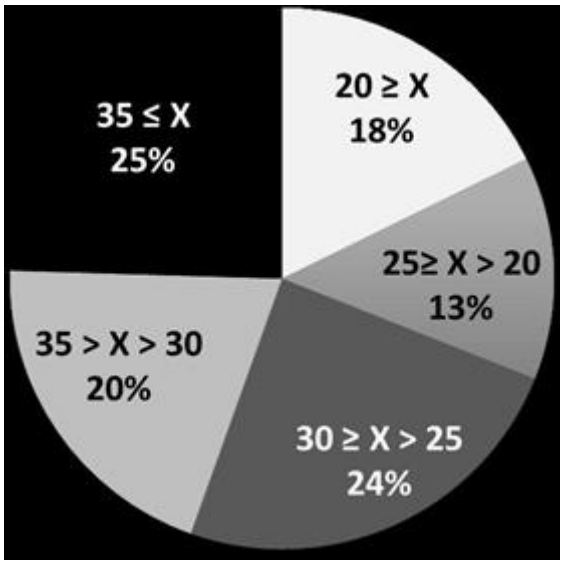

Figure 3. Age analysis of Case group (mothers of DS suffering children) (X=Age).

importance. Since polymorphisms are valuable markers in recognition of some genetic disease, the current study attempted to find a link between mother's genotype and giving birth to a DS child.

Di- or tri-methylation of K9 in H3 histones of heterochromatin regions of centromere has an important role in accurate segregation of sister chromatids during anaphase (20-22). As histones are methylated, DNA methylating enzymes are also recruited to the zone. Methylated DNA makes nucleosomes more stable (3). Electrostatic connection between histones and DNA in regions containing repeated DNA such as centromere, results in DNA compaction and heterochromatiniza-tion. DNA stability is the consequence of coordinate epigenetic processes of DNA and histone methylation and heterochromatinization. Hypomet-hylation of DNA at centromere region leads to the decrease of its density. This hypomethylation may be due to low levels of methyl group donors and

Table 1. Number and allelic frequency of BHMT gene polymorphisms (G742A) of the mothers of DS suffering children

Genotype	Case Group	Control Group
	N (%)	N (%)
GG	17(37.78)	19(63.33)
GA	23(51.11)	1(3.33)
	Allele Frequency	
A	37%	20%
G	63%	83%

results in chromosome instability and disorders such as deletion, translocation, and chromosomal missegregation (2, 6, 9, 23). Impaired homocysteine metabolism induced by genetic polymorphisms of metabolic enzymes, prone the individual to chromosomal damages and acts as a maternal risk factor for giving birth to DS child (11). Early-onset of meiosis activities inside oocytes makes them more vulnerable and missegregation is more probable when an additional factor takes part. Therefore, assuming the same possibility for chromosomal damages in all mothers, the chance of chromosomal instability and missegregation and giving birth to DS children is higher for those having an additional factor such as unfavorable genetic polymorphism and/ or hypomethylation. The results of the present study indicate that the chance of giving birth to DS children is independent from mothers' age. This was also confirmed previously by Migliore et al. (7). Thus, giving birth to DS child is possible in all ages and can be reinforced by genetic polymorphisms. Several studies indicate that polymorphisms of genes of folic acid pathway such as MTR, MTHFR, BHMT are involved in hypomethylation of DNA and chromosomal nondisjunction (2, 4-6, 8, 11, 24). The effect of BHMT gene on remethylation of homocysteine in SAM synthesis pathway was previously reported in Brazilian population (11, 12). Thus, our finding is in agreement with those studies.

Accurate recognition of cellular and molecular events and biochemical pathways which promote maternal chromosome nondisjunction is highly important. It is also highly important to understand the complex interactions among genetic polymorphisms, environmental factors (such as dietary), and epigenetic processes. Such information can be used in genetic conselling. Further extension of this study to a larger population and other ethnicities is of critical importance.

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Conflict of interests

The authors declared no conflict of interests.

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