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Analysis of occurrence of *MTRR* gene polymorphism in Down syndrome and other intellectually disabled children

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ABSTRACT

The 5-methylenetetrahydrofolate homocysteine methyltransferase reductase (MTRR) is a key enzyme involved in folate metabolism. Defect in folate metabolism can lead to abnormal DNA synthesis, repair and methylation which lead to abnormal DNA replication and segregation. The most common polymorphism of *MTRR* genec.66A > G was analyzed in 30 Down syndrome (DS), 30 intellectually disabled (ID) and 60 control children of Gujarat using PCR-RFLP. The results of the present study revealed negative association between *MTRR* c.66A > G and DS using all genetic models except recessive model. Significant positive association was observed between *MTRR* c.66A > G and ID by using dominant, co-dominant and recessive models.

1. Background

Intellectual disability (ID), also known as mental retardation (MR), is one of the most frequent and disabling neurological impairments with major implication for nation's health, education and community services. The World Health Organization (WHO) defined ID as intellectual functioning significantly lower than average with an Intelligence Quotient (IQ) less than or equal to 70 and poor adaptive abilities in at least two of the subsequent areas- self-care and guidance. Down syndrome (DS) is the most common genetic cause of intellectual disability attribute to the 3 copies of chromosome 21. Trisomy 21 occurs due to abnormal segregation during meiosis, mainly by maternal non-disjunction in 95% of cases.

Folate plays a significant role in complex and essential metabolic pathways such as DNA repair, cellular methylation reactions and biosynthesis of nucleotides. The donation of carbon atoms for maintenance of DNA methylation patterns involves an integrated action of many gene products and other important micronutrients which are obtained through the diet, like, vitamin B6, vitamin B12, zinc and methionine (Santos-Reboucas et al., 2008). Defect in folate metabolism can lead to intellectual disability, characteristic facial features and delayed in physical growth (Weijerman et al., 2010). Methionine synthase reductase (MSR) enzyme is encoded by 5-methyltetrahydrofolate-homocysteine methyl transferase reductase (*MTRR*) gene (Doolin et al., 2002). Methionine synthase reductase is one of the key enzymes involved in folate metabolism. MTRR activates 5-methylenetetrahydrofolate homocysteine methyl transferase (MTR) which regenerates methionine from methionine. Vitamin B12 acts as a cofactor in conversion of homocysteine to methionine. Oxidation of cobalamin (cofactor) inactivates methionine synthase. For conversion of inactive form of MTR into active form, S-adenosyl methionine (SAM) is used as a methyl donor. This reaction requires the reductive methylation of cobalamin and is catalyzed by MTRR (Victorino et al., 2014).

MTRR c.66A > G (Substitution of guanine in place of adenine at 66th nucleotide position) is the most common *MTRR* gene polymorphism. *MTRR* c.66A > G leads to MSR p.Ile22Met (substitution of methionine in place of isoleucine at 22nd amino acid position in methionine synthase reductase; O'Leary et al., 2002). Genetic polymorphism may interfere with folate homeostasis, cellular methylations and can leads to DNA strand breakage, DNA methylation, abnormal DNA synthesis, impaired DNA repair and neurological damages (Desai and Chauhan, 2016, 2017). *MTRR* c.66A > G may alter the normal MSR activity due to which it might interfere in normal rate of conversion of homocysteine to methionine. Many studies suggested that *MTRR* c.66A > G can be a potential risk factor for Down Syndrome (Scala et al., 2006; Brandalize et al., 2010; Hobbs et al., 2000; Pozzi et al., 2009). To the best of our knowledge many studies were performed on mothers of children with Down syndrome, whereas present

Abbreviations: ID, Intellectual Disability; WHO, World Health Organization; IQ, Intelligence Quotient; DS, Down Syndrome; MTRR, 5-Methyltetrahydrofolate-Homocysteine Methyl transferase Reductase; MTR, 5-Methylenetetrahydrofolate Homocysteine Methyl transferase; SAM, S-Adenosyl Methionine

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study was performed on children with Down syndrome and intellectual disability.

2. Methodology

2.1. Samples

The present study was ethically approved by S. G. Patel Ayurveda Hospital and Maternity Home. Blood samples of 60 cases (30 Down syndrome, 30 idiopathic intellectual disability) and 60 normal healthy children (control) from different regions of Gujarat, India were collected in Potassium-EDTA vacutainer. The blood samples were collected from special schools of intellectually disabled children and normal schools located at different areas of Gujarat. The consent forms were signed by the parents of the children.

2.2. Molecular analysis

DNA was isolated from 120 blood samples using standard phenol-chloroform method (Shams et al., 2011). PCR amplification was done by making final reaction volume of 25 µl. In each tube 12.5 µl of 2× PCR master mix, 1 µl of forward primer, 1 µl of reverse primer, 5.5 µl of distilled water and 5 µl of genomic DNA (20 ng/µl) was added. The PCR primers were used according the Scala et al. (2006) (forward: CAG CAG GGA CAG GCA AAG GCC ATC GCA GAA GAC AT and reverse: CTG GTG ATA TCT TAC TAT ACC ATA TGA ACA AAC AC).

PCR amplification conditions included: initial denaturation at 94 °C for 4 min, denaturation at 94 °C for 1 min, annealing at 59 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 7 min for a total of 35 cycles. The PCR products were confirmed on 1.5% agarose gel prepared in 1× TBE buffer. The PCR products of 254 bp were digested using *Nde* I restriction enzyme. The 10 µl of PCR product, 1.5µl of cut smart buffer, 8.5µl of distilled water and 1 U of *Nde* I restriction enzyme were added in each tube to make the final reaction volume of 20 µl. The reaction mixture was incubated overnight at 37°C in boiling water bath. The digested PCR products were analyzed on 2.5% agarose gel prepared in 1× TBE buffer.

2.3. Statistical analysis

Allele and genotype frequency were calculated for *MTRR* c.66A > G gene polymorphism. The χ^2 square test for the goodness of the fit was used to check the deviation from Hardy-Weinberg equilibrium for case and control were calculated for *MTRR* c.66A > G polymorphism by using the Social Science Statistic web site (http://www.socscistatistics.com/tests/goodnessoffit/Default2.aspx). Odd's ratio was calculated to check association between *MTRR* c.66A > G and Down syndrome/intellectual disability by using MedCalc statistical software (https://www.medcalc.org/calc/odds_ratio.php).

3. Results

The PCR product of *MTRR* gene was of 254 bp confirmed through agarose gel electrophoresis. The wild type allele (A) showed two restriction sites for *Nde* I restriction enzyme at 34th and 231st nucleotide position in PCR product. Therefore, it produced 3 fragments of 196, 34 and 24 bp size after the digestion with *Nde* I restriction enzyme. The polymorphic allele (G) showed 1 restriction site (internal control) for *Nde* I restriction enzyme at 231st nucleotide position in PCR product. Therefore, it produced two fragments of 230 and 24 bp after the digestion with *Nde* I restriction enzyme. The homozygous normal individuals showed 3 bands of 196, 34 & 24 bp fragments; heterozygous showed 4 fragments of 230, 196, 34 & 24 bp fragments and homozygous mutant showed only two bands of 230 & 24 bp fragments after separation on 2.5% agarose gel. The electrophoretic pattern of PCR and RFLP products are shown in Figs. 1 and 2 respectively.

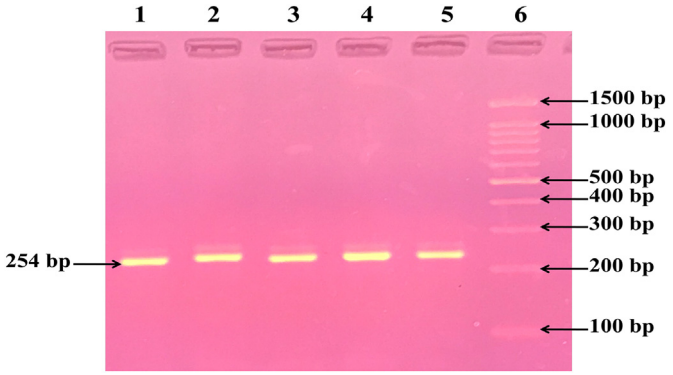

Fig. 1. Electrophoretic pattern of PCR amplified products for *MTRR* c.66A > G polymorphism on 1.5% agarose gel showing band size of 254 bp. In figure, lane 1–5: PCR products of *MTRR* c.66A > G and lane 6: 100–1500 bp DNA ladder.

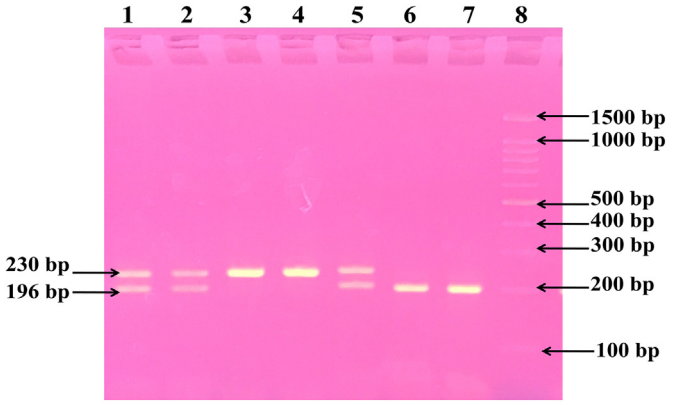

Fig. 2. Electrophoretic pattern of *Nde* I digested products of *MTRR* c.66A > G on 2.5% agarose gel. In figure, Lane 1, 2 & 5 are heterozygous, lane 3 & 4 are homozygous mutant, and lane 6 & 7 are homozygous normal genotype. Lane 8 is 100–1500 bp DNA ladder.

The frequency of wild type (A) allele in intellectually disabled, Down syndrome, and control children were 0.46, 0.45 and 0.38 respectively. The frequency of polymorphic (G) allele in ID, DS and control children were 0.53, 0.55 and 0.6 respectively. The frequency of homozygous normal (AA) genotype in ID, DS and control children were 0.13, 0.23, and 0.41 respectively. Genotype frequency of heterozygous (AG) in ID, DS and control children were 0.8, 0.43 and 0.4 respectively. Genotype frequency of homozygous mutant (GG) in ID, DS and control children were 0.13, 0.33 and 0.41 respectively (Table 1).

The chi-square test for the goodness of fit suggested that all the genotypes were in Hardy-Weinberg equilibrium except in case of intellectual disability (Table 1). The *MTRR* c.66A > G showed positive association with Down syndrome with odd's ratio of 1.43 by using recessive model (AA + GA vs GG) only. While *MTRR* c.66A > G showed positive association with intellectual disability by means of Co-dominant, dominant and recessive model (Table 2).

4. Discussion

Folate is known as vitamin B9 which is necessary for S-adenosyl methionine (SAM) synthesis. Adequate folate intake is required for nucleic acid synthesis and methionine regeneration (de Jonge et al., 2009). MSR is a key enzyme involved in folate metabolism and encoded by *MTRR* gene. *MTRR* c.66A > G polymorphism may reduce the normal enzyme activity and affect the rate of conversion of homocysteine to methionine (Scala et al., 2006).

Intellectual disability (ID) is a most common neuro-developmental disorder of unknown origin. Down syndrome is most common genetic