Prevalence of MTHFR, MTR and MTRR Gene Polymorphisms in Turkish Patients with Nonsyndromic Cleft Lip and Palate
Research Article

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Summary
Objective: To assess the association between the MTHFR gene (A1298C), MTR gene (A2756G) and MTRR gene (A66G) polymorphisms and nonsyndromic cleft lip with or without cleft palate (nsCL/P), we investigated patients in the Turkish population.
Methodology: Analysis of gene polymorphisms was carried out using polymerase chain reactions and restriction enzyme digestions. Our study included 100 patients with nsCL/P and 125 age matched healthy individuals.
Results: We found that MTHFR A1298C polymorphism and MTR A2756G polymorphism are significant risk factors for nsCL/P. The frequency of MTRR A66G homozygotes was higher compared to control group, however the difference was not statistically significant.
Conclusions: We revealed a statistical significant association between the MTHFR A1298C and MTR A2756G polymorphism and nsCL/P.

I. Introduction:
Nonsyndromic cleft lip with or without cleft palate (nsCL/P, MIM 119530) is one of the most common birth defects, with a birth prevalence of approximately 1/1000 in Caucasians (Gundlach & Maus, 2006). The etiology is multifactorial involving both genetic and environmental factors (Carinci et al., 2007; Mitchell et al., 2003; Carinci et al., 2003). A number of studies showed that some genes are associated with nsCL/P; MTHFR, TGFB3, and TGFA (Zhu et al., 2010), p63 (Rinne, Brunner & van Bokhoven, 2007; Barrow et al., 2002), PVRL1 (Turhani et al., 2005). The prevalence rate varies according to geographical origin (Brito et al., 2011), sex (Vanderas, 1987), racial background, ethnicity (Croen, Shaw, Wasserman & Tolarová, 1998) and socioeconomic status (Clark, Mossey, Sharp & Little, 2003).
Folate metabolism may play a role in the mechanism formation of clefting (Brandalize et al., 2007). Previous studies of genes involved folate metabolism; methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR), have suggested an association of polymorphisms with pathogenesis of nsCL/P (Mostowska, Hozyasz & Jagodzinski, 2006; Jagomagi et al., 2010; Chorna, Akopian, Makukh & Fedoryk, 2011; Jugessur et al., 2003). Folate intake (Brandalize et al., 2007) is a very important nutritional substrate for the expression of several genes essential for cellular multiplication and differentiation during embryogenesis. Tetrahydrofolate is the active form of folic acid (Zappacosta et al., 2009; Bhaskara, Murthy & Venkatesh Babu, 2011). MTHFR catalyzes the reduction of 5,10- methylenetetrahydrofolate to 5-methyltetrahydrofolate (Brouns et al., 2008). The MTHFR gene encodes 5,10- methylenetetrahydrofolate reductase (MTHFR) enzyme (EC.1.5.1.20) that consisting of 656 amino acids MTHFR is localized to chromosomal region 1p36.3. The gene consists of 11 exons ranging from 102-432 bp and introns ranging from 250 bp 1.5 kb (Goyette et al., 1994; Reutter et al., 2008; Goyette et al., 1998).

Methionine synthase enzyme (MTR) (EC.2.1.1.13) encoded by the MTR gene, uses the methyl group from 5- methyltetrahydrofolate to remethylate total plasma homocysteine (tHcy) to methionine (Frosts et al., 1995; Barbosa et al., 2008; Yamaji et al., 2009). MTR is localized to chromosomal region 1q42.3-q43 (Chen et al., 1997). Cobalamin (vitamin B12) uses as a cofactor for methylation in folate metabolism. Methionine synthase (MTR) activity, which is inactivated by the oxidation of vitamin B12 which intern depends on methionine synthase reductase (MTRR) (EC.2.1.1.135), encoded by the MTRR gene (Bhaskara, Murthy & Venkatesh Babu, 2011). MTRR is localized to chromosome region 5p15.2-15.3 (Leclerc et al., 1998). MTRR is required for the reductive methylation of vitamin B12 (Wilson et al., 1999).

Polymorphisms of the genes that play a role in folate metabolism, MTHFR, MTR, and MTRR, may be associated with nsCL/P. A common missense mutation in the MTHFR gene converts glutamate to alanine (1298A→C) at 429 amino acid (E429A; rs1801131) which results in a decrease of MTHFR activity, that is more pronounced in homozygous (CC) than heterozygous (AC) state (van der Put et al., 1998). Methionine synthase A2756G is a common nonsynonymous polymorphism in MTR gene resulting in the conversion of an aspartic acid to a glycine at amino acid position 919 (D919G; rs1805087) (Chen et al., 1997; Leclerc et al., 1996). As a result of A2756G polymorphism in MTR gene is an increase in the concentration of plasma homocysteine (Li et al., 1996). Methionine synthase reductase (MTRR) A66G is a common polymorphism in MTRR gene which converts isoleucine to methionine 66 A→G at codon 22 (I22M; rs1801394) (Bhaskara, Murthy & Venkatesh Babu, 2011).

To assess the association between the MTHFR gene (1298A→C), MTR gene (2756A→G) and MTRR gene (66A→G) polymorphisms and nonsyndromic cleft lip with or without cleft palate, we investigated patients in the Turkish population.

**II. Materials and Methods:**

**A. Subjects**

For this study 100 children aged 0-10 years with nsCL/P (the case group), admitted to the Department of Orthodontics of Ankara University Faculty of Dentistry, were selected. Blood samples were collected. The selection
criteria for the nsCL/P group, was no association with any other major malformations. All cases were examined and screened for the presence of association between nsCL/P and other syndromes. Blood samples were collected from this selected group. The control group was comprised of 125 healthy children aged 0-10 years with no reported familial history of orofacial clefts. Mothers of case and control groups were interviewed with same questionnaire.

They were asked to give their age at pregnancy, periconceptional use of vitamin supplements containing folic acid, as well as their smoking (number of cigarettes per day; 0, 1-5 >5) and alcohol consumption (number of drinks per week; 0, 1-2, >2) (Table 1). Study approval was obtained from the Ankara University Faculty of Dentistry, Clinical Research Ethics Committee.

<p>| Table 1: Characteristics of mothers of the case and control groups |
|---------------------------------|----------------|-----------------|------------|</p>
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Case mothers</th>
<th>Control mothers</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤20 years</td>
<td>21 (0.21)</td>
<td>18 (0.14)</td>
<td>-</td>
</tr>
<tr>
<td>20-35 years</td>
<td>69 (0.69)</td>
<td>81 (0.65)</td>
<td>-</td>
</tr>
<tr>
<td>&gt;35 years</td>
<td>10 (0.10)</td>
<td>26 (0.21)</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin supplements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>containing folic acid (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>55 (0.55)</td>
<td>87 (0.70)</td>
<td>1</td>
</tr>
<tr>
<td>Less than daily</td>
<td>45 (0.45)*</td>
<td>38 (0.30)</td>
<td>0.53 (0.31-0.92)</td>
</tr>
<tr>
<td>Maternal smoking/day (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>65 (0.65)</td>
<td>79 (0.63)</td>
<td>1</td>
</tr>
<tr>
<td>1-5</td>
<td>20 (0.20)</td>
<td>15 (0.12)</td>
<td>0.62 (0.29-1.30)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>15 (0.15)*</td>
<td>6 (0.05)</td>
<td>0.33 (0.12-0.90)</td>
</tr>
<tr>
<td>Maternal alcohol consumption/week (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>70 (0.70)</td>
<td>82 (0.66)</td>
<td>1</td>
</tr>
<tr>
<td>1-2</td>
<td>21 (0.21)</td>
<td>13 (0.10)</td>
<td>0.53 (0.25-1.13)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>9 (0.09)</td>
<td>5 (0.04)</td>
<td>0.47 (0.15-1.48)</td>
</tr>
</tbody>
</table>

N, number; OR, odds-ratio; (95%) CI, confidence interval *P<0.05
B. DNA Extraction
Genomic DNA was isolated from peripheral blood samples by conventional phenol-chloroform (Merck, Germany) method using proteinase K (MBI Fermentas, Lithuania). Peripheral blood samples collected in test tubes containing EDTA.

C. Analysis of Polymorphisms
MTHFR gene 1298A→C polymorphism
The genetic analysis of the MTHFR 1298A→C polymorphism was analyzed by PCR-RFLP using forward primer 5’- CTT TGG GGA GCT GAA GGA CTA CTA C-3’ and reverse primer 5’-CAC TTT GTG ACC ATT CCG GTT TG -3’. PCR was carried out in a total volume of 50 μl containing 200 ng of template DNA, 1 U Taq polymerase (Fermentas, Lithuania), 1X (NH4)2SO4 buffer with, 10 pmol of each primer, 0.2 mM of all 4 dNTPs (Fermentas, Lithuania), and 1.5 mM MgCl2. The PCR conditions were, 5 min at 95°C, followed by 37 cycles of 30 s at 95°C, 30 s at 51°C and 30 s at 72°C, with a 10-min elongation step at 72°C at the end of each cycle. A 163 bp fragment was amplified by PCR. The PCR products were electrophoresis in a 2% agarose gel containing ethidium bromide (Figure 1).

![Figure 1.](image)

The 163 bp PCR product (10 μl) was digested with the restriction enzyme FastDigest Mbol (Fermentas, Lithuania) at 37 °C for 5 minutes. DNA fragments were separated by electrophoresis on a 3% agarose gel stained with ethidium bromide at 80 V for 1 hour and visualized under UV light in a transilluminator.

The wild type 1298 A/A genotype resulted in 5 fragments of 56, 31, 30, 28 and 18 bp. The heterozygous 1298 A/C genotype produced 6 fragments of 84, 56, 31, 30, 28 and 18 bp, whereas the homozygous 1298 C/C resulted in 4 fragments of 84, 31, 30, and 18 bp (Figure 2).
Figure 2. MTHFR A1298C polymorphism established by PCR digestion by Mbol. M, 100 bp ladder molecular weight marker. Wild type (1298AA, 56, 31, 30, 28 and 18 bp, lane 8), homozygous type (1298CC, 84, 31, 30, and 18 bp, lane 2), heterozygous type (1298AC, 84, 56, 31, 30, 28 and 18 bp, lane 3-7) and lane 1 is uncut.

D. MTR gene (2756A→G) polymorphism

Determination of the MTR gene 2756A→G polymorphism was carried out by polymerase chain reaction (PCR) amplification, followed by HaeIII (Fermentas) restriction enzyme digestion at 37 °C for 5 minutes and were detected the fragments on 3% agarose gel. Forward primer 5'-GGT GTG TTC CCA GCT GTT AGA TG-3' and reverse primer 5'-GAC ACT GAA GAC CTC TGA TTT GAA C-3' were used for DNA amplification. A 265 bp fragment was amplified by PCR (Figure 3). The 265 bp PCR product was digested with the restriction enzyme FastDigest HaeIII (Fermentas, Lithuania). DNA fragments were separated by electrophoresis on a 3% agarose gel. Heterozygous 2756AG produced 3 fragments of 265, 180 and 85 bp and homozygous 2756GG produced 2 fragments of 180 and 85 bp (Figure 4).

Figure 3. PCR products of MTR gene appearance in the 2% agarose gel. M, 100 bp ladder molecular weight marker. Lane 1-6, 265 bp PCR products.
Figure 4. MTR A2756G polymorphism established by PCR digestion by HaeIII. M, 100 bp ladder molecular weight marker. Wild type (2756AA, 265bp, lane 1,3), homozygous type (2756GG, 180 and 85 bp, lane 4), heterozygous type (2756AG, 265, 180 and 85 bp, lane 2,5).

MTRR gene (66A→G) polymorphism

The genetic analysis of the MTRR 66 A→G polymorphism was analyzed by the polymerase chain reaction PCR-RFLP using forward primer 5’-GCA AAG GCC CAT CGC AGA AGA CAT-3’ and reverse primer 5’-GTG AAG ATC TGC AGA AAA TCC ATG TA -3’. A 66 bp fragment was amplified by PCR (Figure 5).

The 66 bp PCR product was digested with the restriction enzyme FastDigest NdeI (Fermentas, Lithuania) at 37 °C for 5 minutes. The PCR fragment of 66 bp remains uncut in the presence of the G (methionine) allele, but is digested into fragments of 44 and 22 bp in the presence of the A (isoleucine) allele (Figure 6).

Figure 5. PCR products of MTRR gene appearance in the 2% agarose gel. M, 100 bp ladder molecular weight marker. Lane 1-6, 66 bp PCR products
I. Statistical Analysis

Comparisons of genotype and allele frequencies between nsCL/P cases and control groups were calculated. To assess the deviation from Hardy-Weinberg equilibrium, \( \chi^2 \) test was used. Statistical significance was accepted at \( p<0.05 \). The odds ratios (OR) and 95% confidence interval (CI) were calculated and were assessed between MTHFR A1298C, MTR A2756G, MTRR A66G polymorphisms and nonsyndromic cleft lip with or without cleft palate.

A. Vector design

Genotype distribution of the MTHFR A1298C, MTR A2756G, MTRR A66G polymorphisms among nsCL/P cases and controls are shown in Table 2. Sixty-one of 100 case group had the AC genotype and 14 were homozygous in a MTHFR A1298C gene. In the control group (n=125), 84 were heterozygous. The frequency of the C allele in the nsCL/P case group and the control group were 0.44 and 0.18 respectively (Table 3).

III. Results

Genotype distribution of the MTHFR A1298C, MTR A2756G, MTRR A66G polymorphisms among nsCL/P cases and controls are shown in Table 2. Sixty-one of 100 case group had the AC genotype and 14 were homozygous in a MTHFR A1298C gene. In the control group (n=125), 84 were heterozygous. The frequency of the C allele in the nsCL/P case group and the control group were 0.44 and 0.18 respectively (Table 3). There was a statistically significant difference between genotype frequencies of MTHFR A1298C polymorphisms in nsCL/P cases compared with controls (\( p=0.0007 \), odds ratio=22.4 (2.77-181), 95% CI).

Fifteen of case group had the heterozygous (AG) and 7 were homozygous in MTR A2756G gene. In the control group (n=125), 47 were heterozygous and 7 were homozygous. The frequency of AG genotype was significant compared to controls (\( p=0.0004 \), OR=0.29 (0.15-0.56), 95% CI). The frequency of G alleles was statistically significant in nsCL/P cases compared to controls (\( p=0.009 \)).
Table 2: Genotype distribution of the MTHFR A1298C, MTR A2756G, MTRR A66G Polymorphisms among nsCL/P cases and controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control group (n=125) (%)</th>
<th>Case group (n=100) (%)</th>
<th>OR (%)</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1298A→C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>40 (0.32)</td>
<td>25 (0.25)</td>
<td>1</td>
<td></td>
<td>Ref.</td>
</tr>
<tr>
<td>AC</td>
<td>84 (0.67)</td>
<td>61 (0.61)</td>
<td>1.16 (0.64-2.11)</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>1 (0.008)</td>
<td>14 (0.14)</td>
<td>22.4 (2.77-181)</td>
<td>0.0007</td>
<td></td>
</tr>
<tr>
<td>MTR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2756A→G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>71 (0.57)</td>
<td>78 (0.79)</td>
<td>1</td>
<td></td>
<td>Ref.</td>
</tr>
<tr>
<td>AG</td>
<td>47 (0.38)</td>
<td>15 (0.15)</td>
<td>0.29 (0.15-0.56)</td>
<td>0.0004</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>7 (0.05)</td>
<td>7 (0.07)</td>
<td>0.91 (0.30-2.72)</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>MTRR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>66 A→G</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>13 (0.10)</td>
<td>14 (0.14)</td>
<td>1</td>
<td></td>
<td>Ref.</td>
</tr>
<tr>
<td>AG</td>
<td>107 (0.86)</td>
<td>72 (0.72)</td>
<td>0.62 (0.28-1.41)</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>5 (0.04)</td>
<td>14 (0.14)</td>
<td>2.6 (0.73-9.26)</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

N, number; OR, odds-ratio; (95%) CI, confidence interval
*P<0.05
Table 3: Allelic frequencies of the MTHFR A1298C, MTR A2756G, MTRR A66G polymorphisms among nsCL/P cases and controls

<table>
<thead>
<tr>
<th>Allelic frequencies</th>
<th>( \chi^2 )</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1298A</td>
<td>1298C</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>0.82</td>
<td>0.18</td>
</tr>
<tr>
<td>Case group</td>
<td>0.56</td>
<td>0.44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allelic frequencies</th>
<th>( \chi^2 )</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2756A</td>
<td>2756G</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>0.94</td>
<td>0.05</td>
</tr>
<tr>
<td>Case group</td>
<td>0.86</td>
<td>0.14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allelic frequencies</th>
<th>( \chi^2 )</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>66A</td>
<td>66G</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>0.53</td>
<td>0.47</td>
</tr>
<tr>
<td>Case group</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

The frequency of MTRR 66 A→G homozygous was higher compared to control group (OR=2.6 (0.73–9.26), 95% CI, p=0.23), but the difference was not statistically significant. The frequency of G alleles was similar nsCL/P compared to control group (p=0.50), but the differences in frequencies were statistically insignificant.

**IV. Discussion:**

Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is a common birth defect, nsCL/P has a complex etiology including interaction of environmental and genetic factors (Stuppia et al., 2011).

The MTHFR, MTR, MTRR genes were chosen for our study, because these genes are important for catalytic interaction with folate, homocysteine, and cobalamin during embryogenesis (Brouns et al., 2008; Barbosa et al., 2008; Wilson et al., 1999).

There is no previous research on the prevalence of the MTHFR 1298A→C, MTR 2756A→G, MTRR 66A→G polymorphisms with nsCL/P in Turkey, to the best of our
knowledge. Our study is the first report which investigates nsCL/P patients in order to identify associations with MTHFR A1298C, MTR A2756G, MTRR A66G polymorphisms in Turkish patients with nsCL/P and determines frequency for the Turkish population.

MTHFR gene plays an important role in the mechanism formation of clefting. MTHFR gene has two major functional polymorphisms that are C677T (A222V; rs1801133) and A1298C (glutamate to alanine). These polymorphisms have been associated with nsCL/P (Jugessur et al., 2003; van der Put et al., 1998; Zhu et al., 2010). We had previously reported statistical association between the MTHFR C677T gene polymorphism and nsCL/P (p= 0.0004) (Aslar, Özdiler, Altuğ & Taştan, 2013).

The present report has showed that there was a statistically significant difference between genotype frequencies of MTHFR 1298A→C polymorphisms in nsCL/P cases compared with controls. 677 C →T and 1298A→C polymorphisms cause a decrease in the MTHFR enzyme activity (Weisberg, Tran, Christensen, Sibani & Rozen, 1998). MTHFR enzyme is important for generating the active form of folate that need of cells. Folate is required in the form of 5-methyl-tetrahydrofolate for the remethylation of homocysteine in the DNA methylation pathway (Martinelli et al., 2001; Blanton et al., 2011).

At the divergence between populations there are role of folic acid deficiency. Some of studies have shown that folic acid has effected on reducing risk of nsCL/P (Jugessur et al., 2003; van Rooij et al., 2003). Chevrier et al. was found the interaction between fetal and maternal MTHFR gene and maternal folic acid intake in France (Chevrier et al., 2007).

Comparison of women that used daily vitamin supplements containing folic acid use provided evidence that the risk of nsCL/P was increased in the offspring the women taking daily vitamin (p=0.02). This study has supported genetic and environmental interaction between maternal periconceptional use of folic acid supplement and the risk of delivering nsCL/P offspring.

The association between the 1298A→C polymorphism in the MTHFR gene and nsCL/P are controversial in several populations. Jagomagi et al. (included 153 patients) have shown an association between MTHFR A1298C polymorphism and nsCL/P (p=0.0067) (Jagomagi et al., 2010). Similarly, the study conducted by Jugessur et al. were found differences between the cases and controls (Jugessur et al., 2003). We found that MTHFR A1298C polymorphism is a significant risk factor for nsCL/P in Turkey (p=0.0007). These studies supported that MTHFR A1298C polymorphism played a major role in the nsCL/P etiology. However, there is no association in some different populations. Sözen et al. (Sözen, Tolarova & Spritz, 2009), Brandalize et al. (Brandalize et al., 2007), Mills et al. (Mills et al., 2008), Blanton et al. (Blanton et al., 2011) researched with nsCL/P cases and found no differences between the cases and controls.

Maternal smoking in the periconceptional period plays a role in the pathogenesis of nsCL/P. About 4000 compounds have been determined in tobacco smoke that are include aromatic and heterocyclic amines, which can cause metabolic activation and DNA damage (Hein, 2002). In the study performed by Honein et al. were shown association between maternal smoking and nsCL/P (OR=1.3; 95% CI = 1.0-1.6) (Honein et al., 2007).

The number of cigarettes smoked per day (0, 1-5, >5) was comparable between mothers of the case and control groups. Maternal smoking (number of cigarettes per day >5) in the periconceptional period increased the risk
of nsCL/P (p=0.04). Our results have shown that maternal smoking was associated with nsCL/P.

Alcohol consumption inhibits to folate metabolism, and decreases the levels of serum folate (Hillman & Steinberg, 1982; Giovannucci, 2004). Molina-Solana et al. determined that there was an association between maternal alcohol and CL/P (OR 1.28; 95% CI = 0.98–1.66) (Molina-Solana et al., 2013). Maternal alcohol consumption was not statistically significant related to risk of nsCL/P (OR(1-2 drinks, week) =0.53 (0.25-1.13 and OR(>2 drinks, week) =0.47 (0.15-1.48).

Methionine synthase enzyme (MTR) (EC.2.1.1.13) encoded by the MTR gene, uses the methyl group from 5-methyltetrahydrofolate for remethylation of tHcy to methionine (Frosst et al., 1995). The MTR gene converts aspartic acid to a glycine substitution (2756A→G) at 919 amino acid position. MTR 2756A→G polymorphism is located in a domain of the protein that interacts with S-adenosylmethionine (SAM) and auxiliary proteins which are required for reductive methylation. Therefore, MTR 2756A allele could impair the binding of SAM or proteins (Harmon, 1999). We found that in the presence of the AG genotype of the MTR rs1805087 polymorphism compared with AA genotype was a significant risk factor for nsCL/P (p=0.0004).

Chorna et al. found that the risk of nsCL/P was about a 1.5-fold increase in the cases carrying an AG genotype (Chorna, Akopian, Makukh & Fedoryk, 2011). Mostowska et al. revealed that maternal MTR genotype showed significant differences between cases and controls. They found for case mothers with AG genotype was p=0.017, whereas among mothers with GG genotype were p=0.236 (Mostowska, Hozyasz & Jagodzinski, 2006). Study performed by Blanton et al. observed that MTR A2756G polymorphism is associated with Hispanic families with nsCL/P (Blanton et al., 2011). These results supported that there is an association between MTR 2756A→G polymorphism and nsCL/P, but others not supported. MTR A2756G polymorphism did not show significant differences between nsCL/P cases and controls (Brandalize et al., 2007; Bufalino et al., 2010; Mostowska, Hozyasz, Wojcicki, Dzieglewska & Jagodzinski, 2010; Martinelli et al., 2006).

MTRR that is essential for the maintenance of MTR functions is one of the key regulatory enzymes involved in the folate pathway (Wang, Feng, Qiao & Lv, 2013). Gaughan et al. have reported that in the MTRR A66G polymorphism, wild type (AA) genotype cause increased plasma homocysteine levels compared with homozygous (GG) type (Gaughan et al., 2001), but this was not supported by other researchers (Geisel et al., 2001; O’Leary et al., 2002). No association was found for MTRR A66G polymorphism and nsCL/P cases of Italian ancestry (Martinelli et al., 2006). One study reports, MTRR 66AG genotype was associated with the 5.56-fold increased CL/P risk and for case mothers with 2.6-fold increased risk of delivering a nsCL/P offspring (Chorna, Akopian, Makukh & Fedoryk, 2011). A66G polymorphism which converts isoleucine to methionine substitution could disrupt the binding of MTRR to MTR-cobalamin complex. Therefore, decreasing the level of homocysteine remethylation (Olteanu, Munson & Banerjee, 2002).

In the present study, the frequency of MTRR homozygous was higher compared to control group (OR=2.6 (0.73-9.26), 95% CI, p=0.23), but the difference was not statistically significant. Moreover, MTRR A66G polymorphism does not support the association with the development of nsCL/P cases (Brandalize et al., 2007; Mostowska, Hozyasz, Wojcicki, Dzieglewska &
Our findings showed that there is no evidence for MTRR A66G polymorphism risk of Turkish patients with nsCL/P. Also, these results have supported that MTRR 66A→G polymorphism was not statistically significant.

In conclusion, these findings may be helpful in understanding of MTHFR, MTR, and MTRR gene in the etiology of nsCL/P. This is the first report investigating prevalence of MTHFR A1298C, MTR A2756G, MTRR A66G gene polymorphisms in Turkish patients with nsCL/P.

Additionally, studies in different populations with a larger number of nsCL/P cases may be beneficial to verify these results of MTHFR A1298C, MTR A2756G, MTRR A66G polymorphism.

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