

MTHFR C677T Polymorphism in Indonesian Patients with Oral Cleft

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Abstract

The complex pathogenesis and etiology of orofacial cleft (OFC) include numerous environmental, genetic, and epigenetic contributing factors. Recent studies have implicated the MTHFR C677T polymorphism as a possible risk factor for orofacial cleft. The aim of the present work was to assess the genotype distribution of the MTHFR C677T polymorphism in Indonesian OFC patients and healthy control subjects. The PCR-RFLP technique was used to identify the MTHFR C677T genotypes and alleles in 24 cleft cases and 47 control subjects. A significant association was noted in the distribution of genotypes of MTHFR C677T polymorphism between the cleft cases and the control group. The results showed a more pronounced dominance of the CC genotype (79.2% of cleft cases) and a clearer rarity of the TT genotype (none observed in the cleft cases) than has been generally reported for the same polymorphism elsewhere. In conclusion, an association was found between the MTHFR C677T polymorphism and OFC status in Indonesia. However, the number of included cleft cases was relatively small and the study should be extended to a larger sample.

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Introduction

Orofacial clefts (OFC) are among the most common congenital malformations worldwide, and a potentially increasing health burden for developing Asian countries like Indonesia.^{1,2} A recent report in Indonesia shows orofacial cleft occurring in about 1 per 1000 newborns, compared to the world average of 1 in 500–700 live births.^{3,4}

OFC is a birth defect with an ethio pathogenesis that includes environmental, genetic, and epigenetic contributing and interacting factors.

For this work, the particular interest was

in cleft lip with or without palate (CLP±P) that can occur as a syndromic defect together with other anomalies or as a non syndromic single defect. Oral cleft is characterized by a gap formation in the palatum and or lip because of incomplete fusion between the facial processes.

The face, including the oral cavity and lips, begins to develop in the fourth week of human embryogenesis. Initially paired maxillary processes form the lateral upper lip, and the frontonasal prominence also participates to form the philtrum for upper lip. The fusion is completed by the sixth week, and palatogenesis begins in the fifth week, when the intermaxillary segment is formed. In the sixth to seventh week of embryogenesis, the paired palatal shelves begin to grow in an elevated direction horizontally toward to the midline, to fuse in the twelfth week.^{5,6}

This active period of embryogenesis is also highly vulnerable to environmental impacts, for example, by maternal nutrients, smoking, exposure to teratogens, and in general by any factors that can affect DNA, RNA, and protein

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synthesis and related genetic and epigenetic regulation.^{2,7-9}

Nutritional factors, like sufficiency of folic acid in the maternal diet, are clearly important, but other potential interferences in the orofacial development can take place via the activity of key agents, such as the methylenetetrahydrofolate reductase (MTHFR) enzyme of folate metabolism.¹⁰⁻¹² This enzyme activity can be affected by single nucleotide polymorphisms (SNP) in the corresponding gene, and the C677T (rs1801133) polymorphism of MTHFR has been previously implicated as a risk factor for cleft lip and palate.^{13,14}

The impact is generally attributed to reduced enzyme activity and the impaired conversion of 5,10-methylenetetrahydrofolate (5,10 MTHF) to 5-methyltetrahydrofolate (5-MTHF), which is needed in the remethylation from homocysteine to methionine and to support cellular methylation reactions occurring, for example, in epigenetic regulation during embryonic development.^{2,15-19}

In this way both genetic and epigenetic processes can contribute to oral cleft. The aim of the present work was to assess the genotype distribution of the MTHFR C677T polymorphism in Indonesian patients with OFC and healthy control subjects.

Materials and Methods

The genomic DNA samples had been extracted with the same methods as those applied by Auerkari et.al.²⁰ The orofacial cleft group had 24 samples of genomic DNA, consisting of 18 genomic DNA samples from patients with non syndromic cleft lip and 6 genomic DNA samples from patients with non syndromic cleft lip and palate. The control group included 47 samples of genomic DNA from normal individuals without orofacial cleft. This research was approved by the Ethical Committee in the Faculty of Dentistry, University of Indonesia.

Genomic DNA from each sample was amplified according to the methods of Xue et al²¹, with PCR using the following primers: forward 5'-TGA AGG AGA AGG TGT CTG CGG GA - 3' and reverse 5'- AGG ACG GTG CGG TGA GAG TG - 3'.

The final volume for the PCR reaction was 18µl per micro tube, which contained 10µl

BioMix™Red Bioline, 0.5µL forward primers, 0.5µL reverse primers, 6 µL double distilled water, and 1µL genomic DNA. PCR included an initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30s, annealing at 56°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 7 min.

For RFLP analysis, the amplified fragments were digested by the HinfI restriction enzyme (SibEnzyme Ltd) and the fragments were separated by electrophoresis in 3% agarose gel stained with Gel Red™. The results showed one 198bp band indicating the CC genotype (homozygous wildtype), three bands of 198, 175, and 23bp indicating the heterozygous CT genotype, or two bands of 175 and 23 bp indicating the homozygous mutant TT genotype.

Chi-square (χ^2) testing was used for statistical analysis of the results, assuming a p-value less than 0.05 to indicate a significant difference. Hardy-Weinberg equilibrium (HWE) was used to determine the consistency in distribution of each studied population.

Results

The orofacial cleft group consisted of 18 cases of non syndromic cleft lip (CL) and 6 cases of non syndromic cleft lip and cleft palate (CLP); there were 47 samples in the control group. The genotyping results are presented in Figure 1.

The orofacial cleft group had frequencies for each genotype of 19 (79.2%) for the homozygous wild type (CC) and 5 (20.8%) for the heterozygous (CT). No homozygous variant genotype (TT) was found in the orofacial cleft group. The C allele and T allele frequencies were 89.6% and 10.4%, respectively, in the orofacial cleft group.

The control group has showed frequencies for the homozygous wild type genotype, heterozygous, and homozygous variants of 26 (55.3%), 19 (40.4%), and 2 (4.3%), respectively. The frequencies of the genotype and allele MTHFR C677T polymorphism, along with the percentages for each group, are shown in Table 1.

The Hardy-Weinberg equilibrium test was used to confirm that the genotype distribution for every group remained constant. The entire studied population was consistent with HWE, with the p value of HWE presented in Table 1.

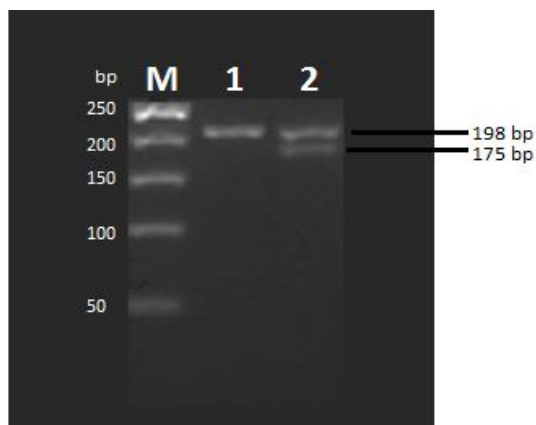


Figure 1. Genotyping for MTHFR C677T polymorphism, digested using the Hinf I (SibEnzyme Ltd) enzyme incubated for the restriction fragment length polymorphism result. M is the DNA marker (50bp ladder); Lane 1 represents homozygous wildtype (CC) at 198bp; Lane 2 represents heterozygous (CT) at 198, 175, and 23bp. No TT homozygous mutant was found.

Table 1. Distribution of the polymorphism gene promoter MTHFR C677T genotype and allelic frequencies for the orofacial cleft group and control group.

SNP	N	Genotypic frequencies			Allelic frequencies		H-W
		CC (%)	CT (%)	TT (%)	C (%)	T (%)	
rs1801133							
Control	47	26 (55.3)	19 (40.4)	2 (4.3)	71 (75.5)	23 (24.5)	$\chi^2=0.41$; db 1; p=0.52
OFC	24	19 (79.2)	5 (20.8)	0	43 (89.6)	5 (10.4)	$\chi^2=0.32$; db 1; p=0.57

The Chi-square test was performed to assess the relation between polymorphic genotypes, which included heterozygous and homozygous variants, and orofacial cleft. The results was statistically significant, with $p=0.048$; $\chi^2=3.893$ (Table 2). Therefore, an association was evident between the polymorphic genotypes

and orofacial cleft incidence. The Chi-square test was also used to analyze the relation between the T allele (the polymorphic allele) and orofacial cleft, and the result showed an association between the T allele and orofacial cleft ($p=0.047$, $\chi^2=3.96$).

Table 2. Chi-square analysis (χ^2) for polymorphic genotype.

Category		Group		χ^2	df	P
		OFC	Control			
Homozygous wildtype	CC	19	26	3.893	1	0.048 ^a
Heterozygous and homozygous polymorphic	CT+TT	5	21			
Total		24	47			

^aStatistically significant

Discussion

The findings in this study indicated the presence of the MTHFR C677T gene polymorphism in the control group and in the orofacial cleft group. In both groups, the homozygous wildtype (CC) was found more frequently than the homozygous polymorphism (TT) and heterozygous polymorphism (CT).

The orofacial cleft group showed no homozygous variant (TT), while the control group

contained two individuals with the homozygous TT variant (4.3%).

Similar studies have been conducted in seven countries, and the genotype distributions varied, as shown in table 2. A study in France found more of the homozygous variant (TT) in the control group than in the orofacial cleft group, indicating a protective role of the T allele.²²

Table 3. Genotype Distribution and allelic rs 1801133 in Orofacial Cleft Studies.

OFC Classification	Region	Allele T (%)		Genotype Frequencies (%)						References	HWE p value Control Group
		Case	Control	Case			Control				
				CC	CT	TT	CC	CT	TT		
CL/P	France	35.1	43.8	44.6	40.5	14.9	39.1	58.7	23.9	Chevrier et al., 2007	0.79
	England	34.9	35.5	40.6	49	10.4	42	45.1	12.9	Little et al., 2008	0.82
	Ireland	33.4	32.7	44.1	45	10.9	44.7	45.1	10.2	Mills et al., 2008	0.34
	China	47.1	39.4	24.6	56.7	18.7	34.7	51.6	13.7	Han et al., 2011	0.24
	South Brazil	36.8	34.5	43	40.4	16.6	45	41	14	Brandalize et al., 2007	0.073
	Venezuela	32.4	32.1	45.3	44.7	10	47.8	47.1	5.1	Sozen et al., 2009	0.07
	Ukraine	37.9	30	36.4	51.5	12.1	44	52	4	Chorna et al., 2011	0.09
CP	France	33.1	43.8	42.4	49.2	8.5	32.1	48.2	19.6	Chevrier et al., 2007	0.79
	England	33.3	35.5	43.5	46.4	10.1	42	45.1	12.9	Little et al., 2008	0.82
	Ireland	31.8	32.7	46.1	44.2	9.7	44.7	45.1	10.2	Mills et al., 2008	0.34
	China	35	49.4	40	50	10	34.7	51.6	13.6	Han et al., 2011	0.24

HWE, Hardy-Weinberg equilibrium Control Group; CC genotype (homozygous wildtype); CT, heterozygous polymorphic; TT, homozygous mutant; CP, cleft palate only; CL/P, cleft lip with or without palate.

The distribution of the homozygous variant in the case and control groups revealed no significant differences in studies from England, Ireland, and South Brazil.^{23,24,25} By contrast, studies from Ukraine¹⁶ and Venezuela²⁶ found higher frequencies of the homozygous variant in the orofacial cleft group than in the control group. Based on this study, the T allele frequencies were higher in the control group than in the orofacial cleft group, with 14.1% discrepancy. This finding was also comparable with the study from France, which also reported T allele

frequencies higher than in the control group. In Venezuela, no difference was evident for T allele frequencies in control and orofacial cleft group. Otherwise study from China found the T allele in orofacial cleft group higher than in the control group.²⁷

This study confirms a significant association between MTHFR C677T polymorphism and orofacial cleft. The statistical analysis indicated a significant association between the T allele and orofacial cleft incidence (p=0.047). The polymorphic genotypes also had

a significant association with orofacial cleft incidence ($p=0.048$). Contrary to this finding, other studies from Ireland²⁴, Ukraine¹⁶, South Brazil²⁵, and Venezuela²⁶ concluded that no association existed between MTHFR C677T polymorphism and orofacial cleft, while a study from China found that MTHFR C677T polymorphism was associated with CL/P, but not with CP.²⁷

However, a recent meta-analysis suggests a slight but significant contribution of the T allele (CT and TT genotypes) to an elevated cleft risk for Asians, but not for Caucasian or African subjects.²⁸ Regional features have also been pointed out in significant differences in the genotype distributions between northern and southern China, showing clearly lower frequencies of the TT genotype for the southern areas but (unlike in the north) again no significant association between the genotypes and cleft cases.¹³

Conclusions

In conclusion, an association was found between the genotype or allele distribution of the MTHFR C677T polymorphism and the OFC status of Indonesian subjects. However, the number of included cleft cases was relatively small and the study should be extended to a larger sample.

Acknowledgements

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Declaration of Interest

The authors report no conflict of interest.

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