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ORIGINAL ARTICLE

Association of a Polymorphism in the Gene Encoding Methylenetetrahydrofolate Dehydrogenase 1 (MTHFD1) 1958G>A with Orofacial Cleft

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ABSTRACT

Objective: To evaluate the possible association of a polymorphism in the gene encoding methylenetetrahydrofolate dehydrogenase 1 (MTHFD1), 1958G>A, with the susceptibility to orofacial cleft in an Indonesian population. **Material and Methods:** A total of 200 stored secondary biological samples from 30 cases of orofacial cleft and 170 unaffected controls were analyzed to determine the polymorphism status at base 1958. The analysis was conducted using the PCR-restriction fragment length polymorphism technique after digestion with the *Msp1* restriction enzyme. The samples were then subjected to agarose gel electrophoresis to investigate the presence or absence of the following fragments: genotype GG, 196, 86 and 40 base pairs (bp); genotype AA, 282 and 28 bp and genotype AG, 282, 196, 86, 40 and 28 bp. The test groups were compared using the Chi-square test. **Results:** The wild-type allele containing 1958G, as well as the genotype GG, were significantly more common in the control group than in the orofacial cleft group. **Conclusion:** The MTHFD1 1958G>A polymorphism was significantly associated with orofacial cleft susceptibility in the tested Indonesian population.

Keywords: Cleft Palate; Cleft Lip; Genetic Variation; Polymorphism, Genetic.

Introduction

Orofacial cleft is a type of congenital malformation of the oral cavity and palate [1]. Previous epidemiologic studies have suggested that the overall prevalence of orofacial cleft is approximately 2 cases per 1000 births. In Indonesia, the corresponding prevalence is approximately 1 case per 1000 live births in 24–59-month-old children [2]. Non-syndromic orofacial cleft is a multifactorial condition attributed to both genetic and environmental factors, including maternal smoking, alcohol consumption and folate deficiency during the peri-conceptual period [3,4]. Particularly, folic acid deficiency has a known impact not only on the manifestation of orofacial cleft, but also on other congenital malformations such as neural tube defects.

In addition to environmental factors, several genes have been identified as potential contributors to the developmental embryogenesis of orofacial cleft. These genes include MTHFD1, a gene that encodes methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) and is thus involved in folate metabolism [5,6]. MTHFD1, which has a chromosomal location of 14q23.3, plays an important role in the generation of one-carbon metabolic derivatives of tetrahydrofolate, including 5,10-methylenetetrahydrofolate cyclohydrolase, 10-formyl tetrahydrofolate and 5,10-methylenetetrahydrofolate dehydrogenase [5].

One previous study revealed the associations of MTHFD1 variants with homocysteine and folic acid levels. Moreover, one common MTHFD1 polymorphism, 1958G>A, can lead to the amino acid substitution of arginine for glutamine, which may reduce the activity and stability of the resulting enzyme and increase the risk of deviations such as orofacial cleft [7]. This polymorphism may also be associated with other craniofacial structural abnormalities, given the expression of MTHFD1 within localized regions in the brain, neural tube, limb buds and tail bud at all stages of embryogenesis [8]. We thereby hypothesized that the MTHFD1 1958G>A polymorphism may be associated with susceptibility to orofacial cleft, and aimed to explore this hypothesis in an Indonesian population.

Material and Methods

Study Design

The study included secondary biological samples of DNA from peripheral blood of 30 cases of orofacial cleft and 170 unaffected controls using the techniques by previous authors [9-11], which were made available from the Oral Biology Laboratory of the Faculty of Dentistry, Universitas Indonesia.

Laboratory Procedures

For DNA isolation, blood samples were inserted into Falcon tubes containing red blood cell anticoagulant (1:3), and the tube was repeatedly inverted for 10 min. The tubes were then centrifuged for 15 min at 1500 rpm at room temperature, supernatant was discarded, and this was repeated 3 times. After adding 2 mL of cell lysis solution (CLS) to the pellet and mixing by inverting, the solution was incubated in a water bath at 37°C for 60 min. Protein precipitation (PP) solution of 1.3 mL was added, vortexed and centrifuged at 40°C/3000 rpm for 5 min. The supernatant was poured into a new tube containing 2.3 mL of cold isopropanol, and the tube was inverted repeatedly until DNA in it appeared white. Then, 1.3 mL of cold 70% ethanol was added to precipitate DNA that was dried by inverting the tube. Washing was repeated two times, and the tube was dried in tilted position. Then, 0.3 mL of Tris-EDTA buffer (TE) was added (adjusted by the amount of DNA), incubated in a water bath at 37°C for 2 h, and finally the solution was transferred into Eppendorf tubes and stored at −20°C.

The following primers were used to amplify a 310-bp PCR product (Figure 1a) containing the target SNP: forward, 5′-CCTGGTTTCCACAGGGCACTC-3′ and reverse, 5′-CCACGTGGGGGCAGAGGCCGGAATACCGG-3′. The PCR amplification protocol comprised an initial denaturation step at 94°C for 7 minutes, followed by 36 cycles of denaturation at 94°C for 60 seconds, annealing at 60°C for 60 seconds and extension at 72°C for 60 seconds.

The PCR products were subject to genotyping via the restriction fragment length polymorphism (RFLP) method after digestion with the restriction enzyme Msp1. After a 16-hour incubation in a water bath at 37°C, the RFLP products were incubated in a thermoblock at 65°C for 20 minutes for enzyme inactivation. The samples were then subjected to agarose gel electrophoresis to investigate the presence or absence of the following fragments: genotype GG, 196, 86 and 40 base pairs (bp); genotype AA, 282 and 28 bp and genotype AG, 282, 196, 86, 40 and 28 bp (Figure 1b).

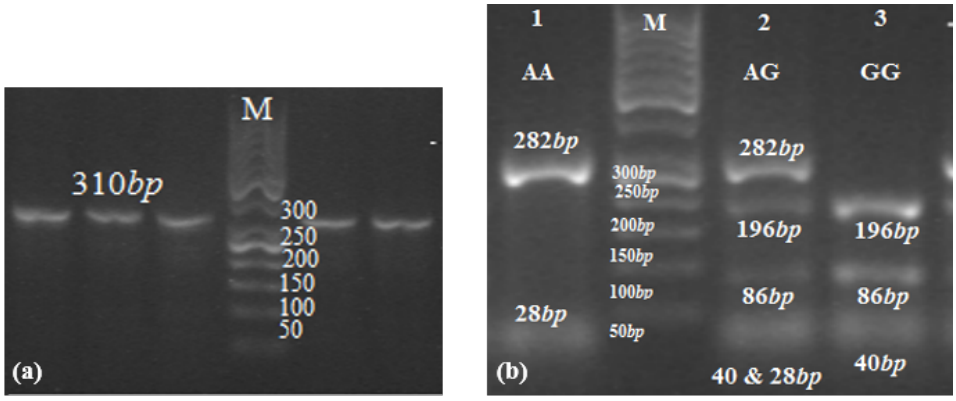

Figure 1. Visualization of a) the 310-bp PCR product in all lanes except M, which contains a 50-bp reference ladder, and b) MTHFD1 1958G>A polymorphism genotypes AA (lane 1), AG (lane 2) and GG (lane 3).

Data Analysis

The test groups were compared using the Chi-square test. SPSS software (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. A p-value of <0.05 was considered to indicate a statistically significant difference.

Ethical Clearance

The protocol was subject to the Ethical approval no.17/Ethical Exempted/FKGUI/XII/2015, protocol No. 01560615. All samples originated from native Indonesian patients who resided mainly on the island of Java.

Results

Table 1 presents the distributions of genotypes and alleles of the MTHFD1 1958G>A polymorphism in the orofacial cleft and control groups. The GG genotype (wild type) and G allele were clearly dominant in the control group, but appeared at significantly reduced frequencies in the case group. The genotype distribution of Table 1 for the case group is consistent with the Hardy-Weinberg equilibrium (HWE) (p=0.543), but the same is not true for the control group (p=<0.001).

Table 1. Distributions of genotypes and alleles associated with the MTHFD1 1958G>A polymorphism in test groups with and without orofacial cleft.

Variables	Orofacial Cleft		p-value
	Yes N (%)	No N (%)	
Genotype			
GG	2 (6.7)	78 (45.9)	0.001
AA	14 (46.7)	43 (25.3)	
GA	14 (46.7)	49 (28.8)	
Total	30 (100.0)	170 (100.0)	
Allele			
G	18 (30.0)	205 (60.3)	0.001
A	42 (70.0)	135 (39.7)	
Total	60 (100.0)	340 (100.0)	

Discussion

Our study of samples from an Indonesian population suggested a significant association of the MTHFD1 1958G>A (rs2236225) polymorphism with orofacial cleft (p=0.001), consistent with the results from previous studies conducted in India and China [6,12]. In contrast to studies of Asian populations, most previous studies involving European (Norwegian, Italian, and Polish) and South American (Brazilian) populations indicated no significant association of this polymorphism with the risk of orofacial cleft [13-17], although one study also suggested such an association in a European (Irish) population [18]. More recently, a review including meta-analyses has casting doubts on the reported association when including studies on Asian populations [19]. Another study has confirmed the lacking association in a wider range of European populations from 11 countries [20]. In other words, although the presumed mechanism underlying the effect of this polymorphism on folate metabolism appears reasonable, the associated evidence does not consistently support this association. The possible effects of ethnic origin, as well as the type of oral cleft (lip, palate or both), may warrant further study [4]. The conclusions have been generally consistent for a number of the reviewed studies that have included all these types of nonsyndromic cleft cases, and a wide range of populations with different ethnic origins [19,20]. Nevertheless, the overall coverage may be instructive but by no means complete: for example, the coverage on African and South-East Asian populations remains rather poor. Unfortunately, the number of samples from patients with orofacial cleft available for the present study was too small for a meaningful subgroup analysis.

While the genotype distribution for the tested polymorphism in the case group from 30 samples of orofacial cleft patients was consistent with HWE, the sample was relatively small. With 170 samples, the control group was considerably larger, but not consistent with HWE-. In comparison, in the other Asian (Indian and Chinese) studies that indicated a significant association of the same polymorphism and orofacial cleft, the genotypes of the control group were consistent with HWE [6,12,19]. Therefore, it appears reasonable to suggest an extension of the present study to a larger case group, for example classified according to the type of cleft, and to extend the comparison to another control group, both from the same ethnic origin.

Other polymorphisms of MTHFD1 than 1958G>A have been previously considered for association with oral cleft susceptibility. In particular, the polymorphisms 31136A>G (rs2357694) and 18913T>C (rs8006686) of MTHFD1 have shown no significant association so far [12], and the same was the case for the polymorphisms rs1950902, rs10813 and rs17857382 in another study [16]. In parallel, other genes encoding enzymes of the folate metabolism, such as MTHFR, MTR and MTRR, and their polymorphic variants, have

been addressed for significance in previous studies, often in studies together with MTHFD1 [13,14,16,17]. Variants with indicated association to oral cleft include e.g. MTHFR rs2274976 [16,17] and with more controversial results, rs1801133 (C677T) of the same gene [17,21,22].

Although individual polymorphisms may exhibit little or weak association with oral cleft, it may still exert influence in combination with other specific polymorphisms. Such influence has been indicated also for MTHFD1 [16,19,20].

For studies on the genetic component in acquiring oral cleft, further applied options include sampling of twins or triads for wider comparisons, and looking into details of e.g. lifestyle and environmental features during pregnancy, to explore confounding factors [12,16]. Such details were not available for the source material of the present study, and it is suggested to expand it first by simply increasing the number of comparable samples.

Conclusion

Our study suggests a significant association of the MTHFD1 1958G>A polymorphism with susceptibility to orofacial cleft in an Indonesian population. Nonetheless, the contradictory results produced by other studies may warrant further analyses of the effects of this polymorphism on other aspects, such as the type of cleft.

Authors' Contributions

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YB	https://orcid.org/0000-0001-8288-9234	Conceptualization, Methodology, Investigation and Writing - Original Draft.
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LDS	https://orcid.org/0000-0001-5542-6787	Formal Analysis.

All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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