C677T mutation in the MTHFR gene was not found in patients with frontoethmoidal encephalocele in East Java, Indonesia

Sadewa, Ahmad hamim

(Degree)
博士（医学）

(Date of Degree)
2005-03-25

(Resource Type)
doctoral thesis

(Report Number)
甲3364

(URL)
https://hdl.handle.net/20.500.14094/D1003364

【 90 】

氏　　名・（本　籍）	AHMAD HAMIM SADEWA	（ インドネシア ）
博士の専攻分野の名称	博士（医学）	
学 位 記 番 号	博い第１６４３号	
学位授与の 要 件	学位規則第５条第１項該当	
学位授与の 日 付	平成１７年３月２５日	

【 学位論文題目 】

C677T mutation in the MTHFR gene was not found in patients with frontoethmoidal encephalocele in East Java, Indonesia
(インドネシア東ジャワの前頭骨篩骨脳瘤患者には MTHFR 遺伝子 C677T 変異を認めなかった)

審 査 委 員

主 査　教 授　林　祥剛
教 授　川端　眞人
教 授　千原　和夫

INTRODUCTION

Frontoethmoidal encephalocele (FEE) is a neural tube defect (NTD) characterized by a congenital bone defect in the anterior cranium and herniation of the intracranial mass through the defect. FEE has a wide spectrum from a huge mass to even an absence of mass. It is more frequent in Southeast Asian than in Western populations. Genetic background, maternal nutritional deficiencies, or other environmental factors may facilitate the development of FEE in this population.

The 5,10-methylenetetrahydrofolate reductase (*MTHFR*) is a key enzyme in the folate metabolism. The C677T mutation, which decreases the enzymatic activity and leads to hyperhomocysteinemia, has been reported as a genetic risk factor for a form of NTD, spina bifida. The role of *MTHFR* in the pathogenesis of FEE remains to be clarified.

PATIENTS AND METHODS

A hospital-based survey of FEE patients who were referred to the Department of Neurosurgery and Plastic Surgery, Malang General Hospital, East Java, Indonesia, was conducted. A total of 138 NTD patients was identified during the study period of 1993-2000.

Thirteen FEE patients (5 males and 8 females), and 8 mothers from 11 FEE families were enrolled in *MTHFR* screening after informed consent was obtained. Forty-seven unrelated healthy individuals from Java volunteered in this study as controls.

Genetic screenings of *MTHFR* mutations were performed using a combination of polymerase chain reaction (PCR), denaturing high-performance liquid chromatography (DHPLC) and direct sequencing. The frequencies of MTHFR mutations were compared by use of chi-square tests, including Fisher's Exact Probability Test.

RESULTS

In total, 130 patients with FEE among 138 NTD patients (94.2%) were identified, 77 were males and 53 were females. Morbidity rate of FEE in the service area of Malang General Hospital was calculated to be 1.45×10^{-5}.

Five common substitutions in *MTHFR* were detected: C121T, C677T, C1060T, A1298C, and G1793A. There was no significant difference in the genotype distribution of each nucleotide substitution among patients, mothers and controls. Interestingly, none of the subjects in this study were homozygous for C677T mutation.

In our patients, two affected siblings were born to consanguineous parents (Family A). The elder brother showed facial features typical of this defect whereas younger sister had a small tumor. *MTHFR* genotype analysis showed no differences between affected and unaffected family members.

DISCUSSION

FEE was found in 94.2% of patients with NTD in East Java Province, Indonesia. Ratios of cranial encephalomeningocele to spinal meningocele (32:1) and of FEE to occipital encephalomeningocele (32:1) were higher than those in other populations. Thus, FEE is the most common form of NTD, although annual case of FEE was not verified. The FEE morbidity rate of 1.45×10^{-5} was lower than that reported in Thailand (1.3 to 3.3×10^{-4}). This discrepancy may be attributed to a possible underestimating of FEE frequency in our study. In addition, our survey analyzed communities with and without FEE patients, while Thailand's survey only on the communities with FEE patients. That may be also a reason for the discrepancy.

We found a high morbidity of FEE in the districts where no pollution by any harmful or teratogenic substances had been reported. Besides, two siblings with FEE

were born to consanguineous parents. These results suggested the possibility that genetic factors contribute to the development of FEE.

MTHFR C677T mutation has been reported as a genetic risk factor of spina bifida. For FEE, however, there were neither mutations nor specific genotype distribution among the patients, mothers, and controls for the five mutations and polymorphisms in *MTHFR* (C121T, C677T, C1060T, A1298C, and G1793A). In addition, homozygosity for the C677T mutation of *MTHFR* was not found in patients and mothers. The analysis of family A also showed that they shared similar genotype for the *MTHFR* mutations and polymorphisms. We suggested that *MTHFR* mutations and polymorphisms do not contribute to the development of FEE.

These results supported the idea that genetic risk factors of FEE and spina bifida may be different. In fact, there was no report of FEE and anencephaly, spina bifida or other NTDs occurred in a single patient or in siblings of one family. Compound mutants of the *Alx3* and *Alx4* homeobox genes in mice have been shown to produce craniofacial defects which showed a model suggestive of FEE. The development of FEE may require a set of mutations in independent genes on the analogy of the *Alx3/Alx4* double-mutant mice (If it is the case in human, it is much more complicated to determine the inheritance trait of FEE than a single gene disorder.).

In conclusion, we showed that FEE is the most common form of NTD in East Java, Indonesia. Genetic analysis of 11 affected families suggests that the *MTHFR* gene is not associated with the development of FEE in East Java, although the number of FEE families analyzed in this study was very limited.

論文審査の結果の要旨

受付番号	甲第 1635 号	氏　名	Ahmad Hamim Sadewa
論文題目 Title of Dissertation	C677T mutation in the MTHFR gene was not found in patients with frontoethmoidal encephalocele in East Java, Indonesia インドネシア東ジャワの前頭骨篩骨脳瘤患者には MTHFR 遺伝子 C677T 変異を認めなかった		
審査委員 Examiner	主査 Chief Examiner 林 祥剛 副査 Vice-examiner 川端 善一 副査 Vice-examiner 千原 和夫		
審査終了日	平成 17 年 2 月 9 日		

（要旨は１，０００字～２，０００字程度）

Frontoethmoidal encephalocele (FEE) is a neural tube defect (NTD) characterized by a congenital bone defect in the anterior cranium and herniation of the intracranial mass through the defect. FEE has a wide spectrum from a huge mass to even an absence of mass. It is more frequent in Southeast Asian than in Western populations. Genetic background, maternal nutritional deficiencies, or other environmental factors may facilitate the development of FEE in this population.

The 5,10-methylenetetrahydrofolate reductase (*MTHFR*) is a key enzyme in the folate metabolism. The C677T mutation, which decreases the enzymatic activity and leads to hyperhomocysteinemia, has been reported as a genetic risk factor for a form of NTD, spina bifida. The role of *MTHFR* in the pathogenesis of FEE remains to be clarified.

PATIENTS AND METHODS

Population Study

A hospital-based survey of FEE patients who were referred to the Department of Neurosurgery and Plastic Surgery, Malang General Hospital, East Java, Indonesia, was conducted. A total of 138 NTD patients were identified during the study period of 1993-2000.

Molecular Genetics Study

Thirteen FEE patients (5 males and 8 females), and 8 mothers from 11 FEE families were enrolled in *MTHFR* screening after informed consent was obtained. Forty-seven unrelated healthy individuals from Java volunteered in this study as controls.

Genomic DNA was extracted from 2-5 ml of whole blood from each individual by using a DNA extraction kit, SepaGene (Sanko Junyaku, Tokyo, Japan). For the mutation analysis of the *MTHFR* gene, we used a combination of polymerase chain reaction (PCR), denaturing high-performance liquid chromatography (DHPLC) and direct sequencing.

PCR was carried out in a PC-700 thermal cycler (Astec, Tokyo, Japan). To analyze the entire coding region, all primers were designed on the basis of the intron sequences. Eleven MTHFR exons were amplified using this method.

To form hetero- and homoduplexes of DNA fragments, the mixture of the PCR products of the test and reference subjects was hybridized by heating to 95°C for 5 min and cooling to 25°C at 1.5°C/min. Subsequently, 5 µl of the mixture was analyzed by an automated DHPLC system, the WAVE® Nucleic Acid Fragment Analysis System, equipped with a DNASep® cartridge (Transgenomic, Omaha, NE, USA).

The PCR products of test subjects with heteroduplex peaks on the chromatogram were sequenced on an ABI PRISM 310 DNA sequencer with a BigDye terminator mix (Applied Biosystems).

The frequencies of *MTHFR* mutations were compared by use of chi-square tests, including Fisher's Exact Probability Test.

RESULTS

Population study

During 1993 to 2000, 138 cases of NTD were diagnosed. Of the 138 patients, 130 (94.2%) were diagnosed as having FEE. Of these 130 patients, 77 were males and 53 were females. The male to female ratio was 1.45:1. The ratio of cranial meningocele to spinal meningocele was 32:1, and the ratio of frontoethmoidal to occipital meningocele was 32:1. Thus, FEE is the most common form of NTD in East Java, Indonesia.

The frequency of FEE in the nine districts served by the department was 1.45×10^{-5}. Interestingly, several limited districts in Malang area (Kodya Malang, Malang, and

Kotatip Batu) showed a high morbidity rate of 3.25 $x10^{-5}$, especially Kotatip Batu with morbidity rate of 6.74 $x10^{-5}$.

MTHFR mutations and polymorphisms in FEE patients and their mothers

In total, we detected five nucleotide substitutions, C121T, C677T, C1060T, A1298C, and G1793A, in the coding region of the *MTHFR* gene. These nucleotide substitutions have been reported elsewhere and are recognized as common mutations or polymorphisms in the *MTHFR*. None of the subjects tested in this study were homozygous for T at nucleotide position 677. There was no significant difference in the genotype distribution of each nucleotide substitution between patients and controls, or between mothers and controls.

Study of Family A with two affected siblings

The FEE patients showed a variety of clinical phenotypes, ranging from a huge mass recognized at birth to a tiny mass noted in childhood and adulthood. In an FEE family with four children (Family A), two siblings were affected. Interestingly, the father and mother were identified to be descendants of the first cousins. The elder brother showed facial features typical of this defect whereas the younger sister had a small tumor which became apparent only on crying. None of the family members were affected with FEE, although all of them lived in the same village. *MTHFR* genotype analysis of the affected and unaffected siblings showed heterozygosity for the nucleotides at positions 121, 1060, 1298, and 1793. None of the family members were homozygous for T at nucleotide position 677.

CONCLUSION

This is the first epidemiological study of FEE in East Java, Indonesia, and the result showed that FEE is the most common form of NTD in that area. It has also been reported that FEE phenotype is very different in two affected siblings of Family A. The molecular genetic analysis of 11 affected families, however suggests that the development of FEE is not associated with the *MTHFR* gene, in which related to the development of spina bifida. Thus, this investigation is important from epidemiology and molecular biology point of view, providing additional knowledge on FEE. The researcher, therefore, is recognized to qualify to receive the degree of Ph.D. (Medicine).