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ICAM1 K469E Polymorphism Effect in Gastroschisis Patients

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Objective: To evaluate the association between a common cell-cell interaction variant, *intracellular adhesion molecule 1* (*ICAM1*) K469E, and gastroschisis risk in Indonesia.

Summary of Background Data: Gastroschisis is a congenital disorder characterized by fetal intestines' extrusion outside the body. Several hypotheses have been proposed for gastroschisis, including an impairment in the normal attachment between umbilical cord and umbilical ring.

Methods: A total of 48 infants with gastroschisis and 88 ethnicity-matched controls were involved in this study. The *ICAM1* K469E polymorphism was analyzed using polymerase chain reaction–restriction fragment-length polymorphisms on genomic DNA.

Results: The frequencies of genotypes for *ICAM1* K469E in patients were KK, 30 of 48 (63%); KE, 17 of 48 (35%); and EE, 1 of 48 (2%), whereas their frequencies in controls were KK, 48 of 88 (55%); KE, 33 of 88 (37%); and EE, 7 of 88 (8%). Those frequencies were not significantly different between both groups ($P = 0.37$) with an OR of 1.39 (95% CI, 0.68–2.85). The K469 allele had a frequency of 80% (77 of 96) in patients and 73% (129 of 176) in controls, and the frequency in patients was not significantly higher than that in controls ($P = 0.20$), with an odds ratio of 1.48 (95% confidence interval, 0.81–2.70). In addition, the frequency of *ICAM1* K469 allele in patients with maternal age younger than 25 years was

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not significantly higher than that of patients with maternal age 25 years or older ($P = 0.55$), with an odds ratio of 0.72 (95% confidence interval, 0.25–2.11).

Conclusions: *ICAM1* K469E is not a common susceptibility factor for gastroschisis in Indonesia. A multicenter study with larger number of participants is necessary to clarify these results.

Key words: Gastroschisis – *ICAM1* – K469E – Polymorphism – Indonesia

Gastroschisis (MIM No. 230750) is a congenital disorder characterized by fetal intestines' extrusion outside the body. The prevalence rate of gastroschisis varies among ethnic groups: 5 to 40 per 100,000 live births.[1] Its prevalence has been increasing in the United States and worldwide.[2]

There are several hypotheses about the pathogenesis of gastroschisis, including one involving an impairment in the normal attachment between umbilical cord and umbilical ring.[3] This anatomic defect might be due to a reduced cell deposition and impaired cord attachment at the umbilical ring.[3]

Recently, several studies have demonstrated an association between gastroschisis risk and common variants of the cell-cell interaction gene *intracellular adhesion molecule 1* (*ICAM1*).[4,5] So far, there have been no such reports from the Asian region. Therefore, an additional study is warranted in Asia, particularly in Indonesia, because the *ICAM1* K469E polymorphism frequency differs among ethnic groups,[6] and its effect on gastroschisis has never been determined to date. In this study, we analyzed the *ICAM1* common variant K469E in the Indonesian population both to assess its control frequency and whether this polymorphism shows an association with gastroschisis.

Patients and Methods

Patient samples

We included 48 gastroschisis patients, including 25 male and 23 female patients. We used 88 ethnicity-matched individuals with no diagnosis of gastroschisis as controls. This study was reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Universitas Gadjah Mada (UGM)/Dr Sardjito Hospital (KE/FK/83/EC). Written informed consent was obtained from all parents for this study.

DNA isolation and genotyping

Genomic DNA was extracted from abdominal wall tissue and/or a blood sample of the 48 gastroschisis probands and from the peripheral blood of the 88 control samples, using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The *ICAM1* K469E polymorphism was chosen based on previous evidence.[4] Genotyping of the variant was performed using the polymerase chain reaction–restriction fragment-length polymorphisms (PCR-RFLP) technique (Fig. 1). In brief, the *ICAM1* fragment containing the K469E variant was amplified using forward primer 5′-CCATCGGGGAATCAGTG-3′ and reverse primer 5′-ACAGAGCACATTCACGGTC-3′. Subsequently, the PCR product was digested by endonuclease *Bst*UI restriction enzyme. The E469 allele creates a restriction site for the enzyme.[7] The digestion products were separated on 3% agarose gel and subsequently were visualized by ethidium bromide staining.

Statistical genetic analysis

The χ^2 test was performed to compare the distribution of genotypes and alleles between patient and control groups. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated, with values of $P < 0.05$ considered significant. The PLINK was used for tests of Hardy-Weinberg equilibrium.[8]

Results

The frequencies of genotypes for *ICAM1* K469E in patients were KK, 30 of 48 (63%); KE, 17 of 48 (35%); and EE, 1 of 48 (2%), whereas their frequencies in controls were KK, 48 of 88 (55%); KE, 33 of 88 (37%); and EE, 7 of 88 (8%). Those frequencies were not significantly different between both groups ($P = 0.37$), with an OR of 1.39 (95% CI, 0.68–2.85). The K469 allele had a frequency of 80% (77 of 96) in patients and 73% (129 of 176) in controls, and the frequency in patients was not significantly higher ($P = 0.20$), with an OR of 1.48 (95% CI, 0.81–2.70; Table 1). Furthermore, those genotypes were in Hardy-Weinberg equilibrium ($P = 0.69$).

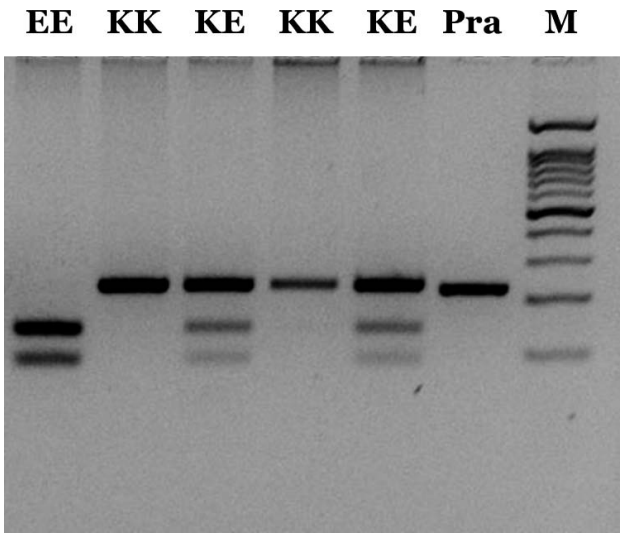

Fig. 1 Restriction analysis of the *ICAM1* common variant K469E. M, marker; Pra, pra-digested PCR.

Then, we compared the observed risk allele frequencies in Indonesian controls with those reported for the 1000 Genomes Project Asian ancestry controls.[6] The *ICAM1* K469 allele (0.73 versus 0.72) had a frequency similar to that in the 1000 Genomes Project Asian ancestry individuals. However, the *ICAM1* K469E showed high variation within Asia, with a range of 0.56 to 0.78 in Japanese and Southern Han Chinese.[6]

Moreover, the K469 allele had a frequency of 78% (47 of 60) in patients with maternal age younger than 25 years and 83% (30 of 36) in patients with maternal age 25 years or older, and the frequency in gastroschisis infants with maternal age younger than 25 years was not significantly higher ($P = 0.55$), with an OR of 0.72 (95% CI, 0.25–2.11; Table 2).

Discussion

In this study, we have analyzed the frequency of the *ICAM1* common variant K469E in an Indonesian population and its associations with gastroschisis. We were unable to find evidence of the genetic effect of *ICAM1* K469E in Indonesian gastroschisis cases. To the best of our knowledge, this is the first study for the association between the *ICAM1* common variant K469E and gastroschisis risk. Our study revealed that *ICAM1* K469E is not a genetic risk for gastroschisis, with a background allele frequency of approximately 73% in Indonesia (Table 1), differing from a previous study.[4] These differences might relate to Indonesian genetic structure ethnicity.[9]

Table 1 The genotypes and allele frequencies of ICAM1 *K469E polymorphism in infants with gastroschisis and controls*[a]

	Frequency, No. (%)		
	Gastroschisis	Controls	OR (95% CI); *P* value
Genotype			
KK	30 (63)	48 (55)	Dominant (KK + KE versus EE) 4.06 (0.49–34.04); 0.16
KE	17 (35)	33 (37)	
EE	1 (2)	7 (8)	Recessive (KK versus KE + EE) 1.39 (0.68–2.85); 0.37
Allele			
K	77 (80)	129 (73)	1.48 (0.81–2.70); 0.20
E	19 (20)	47 (27)	

[a]The gene and variants genotyped, allele frequencies in infants with gastroschisis and controls, the OR, and its 95% CI, with statistical significance values (*P* values), are shown.

Ojeda-Ojeda *et al*[10] demonstrated that *ICAM1* common variant K469E was strongly associated with polycystic ovary syndrome in Spain, but it was not a risk factor for polycystic ovary syndrome in other ethnic groups.[11]

Furthermore, our recent study is another example of differential association of common variants with a disease in different ethnic backgrounds, an example being *MTHFR* polymorphism c.677C>T.[12] This variant has been associated with gastroschisis in our population, but it has not shown an effect on gastroschisis in white individuals.[13] Other examples are *NRG1* common variants rs16879552 and rs7835688. These polymorphisms have been related to Hirschsprung disease in Asian ancestry cases[14–16]; however, they are not associated with Hirschsprung disease in white ancestry patients.[17,18]

Table 2 The genotypes and allele frequencies of ICAM1 K469E in gastroschisis infants with regard to maternal age[a]

	Frequency, No. (%)		
	Maternal age <25 y	Maternal age ≥25 y	OR (95% CI); *P* value
Genotype			
KK	18 (60)	12 (67)	Dominant (KK + KE versus EE) 0.97 (0.91–1.03); 0.43
KE	11 (37)	6 (33)	
EE	1 (3)	0	Recessive (KK versus KE + EE) 0.75 (0.22–2.55); 0.64
Allele			
K	47 (78)	30 (84)	0.72 (0.25–2.11); 0.55
E	13 (22)	6 (17)	

[a]The gene and variants genotyped, allele frequencies in infants with gastroschisis with regard to maternal age, the OR, and its 95% CI, with statistical significance values (*P* values), are shown.

In contrast, *SEMA3* rs11766001 polymorphism is strongly associated with Hirschsprung disease risk by case-control and TDT analyses in white individuals,[17] but its frequency is almost absent in the Indonesian population.[19]

Interestingly, there was racial variability in the outcomes of neonates with gastroschisis.[20] Hispanic gastroschisis neonates showed significantly higher mortality than African American and white infants.[20] Further investigation is necessary to elucidate whether the outcomes of gastroschisis patients are affected by the common variant.

It has been shown that maternal age is a risk factor for gastroschisis.[21] However, our study also showed that the frequency of *ICAM1* K469 allele does not differ between the patients with maternal age younger than 25 years and the patients with maternal age 25 years and older (Table 2).

ICAM1 has been shown to have a role in the leukocytes' and monocytes' adhesion to the activated endothelium. ICAM1 has 5 extracellular domains involving circulatory leukocyte binding sites for recruiting it at the inflammation sites, and it has a tight adhesion with vascular endothelium to form macrophages transformed foam cells.[22] Previous reports revealed the effect of *ICAM1* polymorphism on gastroschisis.[4,5] However, our study was unable to show an association between *ICAM1* K469E polymorphism and gastroschisis risk, implying that the gastroschisis pathogenesis might be affected by several genetic risks, not only *ICAM1* K469E variant. Furthermore, it seems likely that the gene-environment interaction has more impact on gastroschisis pathogenesis than a single genetic etiology.[5] Other concerns regarding our study were statistical power issues and small sample size.

Conclusions

ICAM1 K469E is not a common susceptibility factor for gastroschisis in Indonesia. A multicenter study with a larger number of participants is necessary to clarify these results.

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