

A Novel Susceptibility Locus Near GRIK2 Associated With Erosive Esophagitis in a Korean Cohort

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INTRODUCTION: The male-predominant sex difference through the spectrum of erosive esophagitis to Barrett's esophagus is widely known. We conducted a genome-wide association study (GWAS) stratified by sex for identifying factors that can predict the endoscopically diagnosed erosive esophagitis.

METHODS: Erosive esophagitis was diagnosed by endoscopy and assessed for severity. We identified genetic factors associated with erosive esophagitis that accounted for the sex differences in a cohort of 4,242 participants via a GWAS. After quality control and imputation, genetic associations with erosive esophagitis were investigated by multivariate linear regression in 3,620 subjects. Single-nucleotide polymorphisms (SNPs) with $P < 5.0 \times 10^{-8}$ were considered significant genome wide, and a genetic risk score was constructed for the prediction of erosive esophagitis risk.

RESULTS: Six genome-wide significant SNPs near the GRIK2 gene on chromosome 6 were found to be associated with erosive esophagitis only in male subjects. These were predictive of severity through a genetic risk score ($P < 0.05$), and the findings were validated in a cohort of 622 subjects ($P < 0.05$).

DISCUSSION: This is the first GWAS of erosive esophagitis, and we identified 6 genome-wide significant SNPs in male subjects. These SNPs could help explain the pathogenesis of erosive esophagitis and contribute to the understanding of sex differences. Further genetic investigation could allow for the prediction of high risk for erosive esophagitis and development of new treatment options.

SUPPLEMENTARY MATERIAL accompanies this paper at <http://links.lww.com/CTG/A220>, <http://links.lww.com/CTG/A221>

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INTRODUCTION

Gastroesophageal reflux disease (GERD) develops when the reflux of gastric contents causes symptoms, such as heartburn and acid regurgitation (1,2). GERD symptoms are not perfectly correlated with endoscopic findings, however, with less than 50% of symptomatic patients showing signs of esophageal damage (1,3). According to endoscopic findings, cases with GERD can be classified into erosive esophagitis and nonerosive reflux disease (NERD) (4). Erosive esophagitis, or reflux esophagitis, is defined as an endoscopically proven esophageal mucosal injury above the gastroesophageal junction. Erosive esophagitis with chronic esophageal injury can result in Barrett's esophagus (BE), the replacement of normal squamous epithelium with specific columnar epithelium in the lower esophagus, leading to intestinal metaplasia (5). Although not all mechanisms of esophageal adenocarcinoma (EAC) have been identified, it is generally accepted

that there is a sequential progression from erosive esophagitis to BE and finally to EAC (6).

Although no sex differences have been observed in the overall prevalence of GERD, erosive esophagitis is more common in men, whereas NERD is more common in women (7,8). In addition, BE and EAC are more frequently diagnosed in men, in an extension of pathological GERD (5,9). Because of the biological and physiological differences between men and women, sex affects the susceptibility, progression, and response to treatment for many diseases. It is also believed that men may be at a higher risk of pathogenesis and disease progression of erosive esophagitis (2,10,11).

Previous family and twin studies suggested a genetic influence on GERD development (12,13). Recently, many genome-wide association studies (GWAS) have made an effort to elucidate the genetic architecture of the disease. A recent GWAS identified

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a shared genetic background between GERD and BE and EAC (14). Some studies have identified several loci associated with the development of BE and EAC (15–17). However, no genome-wide significant single-nucleotide polymorphism (SNP) has been found for GERD. Although it is known that erosive esophagitis is more associated with esophageal structure, BE, and EAC than NERD, there has not yet been a GWAS study for the endoscopically diagnosed erosive esophagitis (1).

The conventional GWAS approach is a systematic search of SNPs across the genome to identify a novel genetic variation and find disease-related genes. Recently, several GWAS were conducted stratified by sex because of the reported sex differences in the prevalence and course of disease (18,19). In this study, we conducted a GWAS stratified by sex to investigate genetic factors associated with the endoscopically proven erosive esophagitis.

METHODS

Study subjects

We collected data from 10,349 individuals from the gene-environmental interaction and phenotype cohort, who visited the Seoul National University Hospital, Healthcare System Gangnam

Center, for routine health checkups from 2014 to 2015. Detailed population characteristics and cohort protocols have been previously described (20). From the gene-environmental interaction and phenotype cohort, we retrospectively collected the data of 4,242 individuals aged 50 years and older who underwent upper gastrointestinal endoscopic examination for gastric cancer screening. We divided the enrolled population into 2 groups based on the time of enrollment. Samples donated between January 2014 and April 2015 comprised the discovery cohort (n = 3,620), and those enrolled between May 2015 and December 2015 comprised the replication cohort (n = 622).

Data collection and SNP analysis

All participants donated blood samples for DNA collection and genotyping after informed consent was obtained. Donated blood samples were stored at a biorepository, and genomic DNA was isolated. SNP genotyping was performed by hybridization on an Affymetrix Axiom KORV1.0-96 Array (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's protocol. Genotype data were produced using K-CHIPs designed by the Center for Genome Science, Korea National Institute of Health,

Table 1. Baseline characteristics of the discovery and validation sets according to sex differences

| Male subjects | Discovery set subjects | | | Replication set subjects | | |
|--------------------------|------------------------|--------------|---------|--------------------------|--------------|---------|
| | Control (N = 1,777) | EE (N = 493) | P value | Control (N = 297) | EE (N = 100) | P value |
| Age (yr) | 58.2 ± 6.2 | 58.1 ± 6.0 | 0.733 | 56.8 ± 4.8 | 57.4 ± 4.9 | 0.269 |
| Waist circumference (cm) | 86.5 ± 6.7 | 87.9 ± 6.8 | <0.001 | 87.2 ± 7.1 | 88.7 ± 6.9 | 0.077 |
| Smoking status | | | 0.005 | | | 0.894 |
| Never | 380 (23.0%) | 76 (16.2%) | | 64 (23.8%) | 19 (21.3%) | |
| Ex-smoker | 974 (59.0%) | 293 (62.5%) | | 161 (59.9%) | 55 (61.8%) | |
| Current smoker | 297 (18.0%) | 100 (21.3%) | | 44 (16.4%) | 15 (16.9%) | |
| Alcohol consumption | | | 0.059 | | | 0.682 |
| ≥140 g/wk or ≥20 g/d | 553 (31.1%) | 180 (36.5%) | | 78 (26.3%) | 31 (31.0%) | |
| Medication treatment | | | | | | |
| Diabetes mellitus | 168 (9.5%) | 47 (9.5%) | 1.000 | 33 (11.1%) | 15 (15.0%) | 0.393 |
| Hypertension | 496 (27.9%) | 187 (37.9%) | <0.001 | 91 (30.6%) | 37 (37.0%) | 0.292 |
| Female subjects | Discovery set subjects | | | Replication set subjects | | |
| | Control (N = 1,227) | EE (N = 123) | P value | Control (N = 193) | EE (N = 32) | P value |
| Age (yr) | 57.4 ± 5.7 | 58.1 ± 6.3 | 0.212 | 56.8 ± 4.5 | 58.8 ± 5.7 | 0.027 |
| Waist circumference (cm) | 79.4 ± 7.4 | 82.2 ± 10.2 | 0.003 | 80.6 ± 6.5 | 80.4 ± 8.3 | 0.906 |
| Smoking status | | | 0.971 | | | 0.818 |
| Never | 747 (94.8%) | 76 (95.0%) | | 94 (93.1%) | 19 (95.0%) | |
| Ex-smoker | 23 (2.9%) | 2 (2.5%) | | 5 (5.0%) | 1 (5.0%) | |
| Current smoker | 18 (2.3%) | 2 (2.5%) | | 2 (2.0%) | 0 (0.0%) | |
| Alcohol consumption | | | | | | |
| ≥140 g/wk or ≥20 g/d | 11 (0.9%) | 2 (1.6%) | 0.652 | 1 (0.5%) | 0 (0.0%) | 0.307 |
| Medication treatment | | | | | | |
| Diabetes mellitus | 41 (3.3%) | 9 (7.3%) | 0.048 | 9 (4.7%) | 1 (3.1%) | 0.999 |
| Hypertension | 202 (16.5%) | 31 (25.2%) | 0.020 | 44 (22.8%) | 11 (34.4%) | 0.234 |

EE, erosive esophagitis.

Table 2. Endoscopic finding and erosive esophagitis according to the Los Angeles classification (N = 4,242)

| | n | % |
|---|-----|------|
| Erosive esophagitis | | |
| Overall | 748 | 17.6 |
| Male | 593 | 22.3 |
| Female | 155 | 9.8 |
| Severity of erosive esophagitis (men), n = 593 | | |
| LA grade A | 450 | 75.9 |
| LA grade B | 129 | 21.8 |
| LA grade C or D | 14 | 2.4 |
| Severity of erosive esophagitis (women), n = 155 | | |
| LA grade A | 136 | 87.7 |
| LA grade B | 16 | 10.3 |
| LA grade C or D | 3 | 1.9 |
| Hiatal hernia | | |
| Hiatal hernia without erosive esophagitis (n = 3,494) | 35 | 1.0 |
| Hiatal hernia with erosive esophagitis (n = 748) | 78 | 10.4 |
| BE | | |
| BE without erosive esophagitis (n = 3,494) | 52 | 1.5 |
| BE with erosive esophagitis (n = 748) | 30 | 4.0 |

BE, Barrett's esophagus; LA, Los Angeles.

Korea (4845–301, 3000–3031). Genotyping was performed by DNA Link (Seoul, Korea).

Endoscopic findings

All upper gastrointestinal endoscopies were performed using a conventional white-light videoendoscope (GIF-H260 series endoscopes; Olympus Optical, Tokyo, Japan) by one of the 16 board-certified gastroenterologists. Erosive esophagitis, defined as mucosal breaks above the gastroesophageal junction, was diagnosed through endoscopy. The severity of erosive esophagitis was graded from A to D according to the Los Angeles (LA) classification with Japanese modifications (21). The accompanying findings, such as hiatal hernia, were recorded.

Quality control and statistical analysis

Quality control was performed using PLINK (version 1.07; Free Software Foundation, Boston, MA). We excluded samples with (i) sex inconsistencies, (ii) missing genotype call rate >3%, and (iii) related or cryptically related individuals (identical by descent >0.185). SNPs were excluded if they had (i) missing call rate >5%, (ii) minor allele frequency <1%, and (iii) *P* value of the Hardy-Weinberg equilibrium test <10⁻⁵. SHAPEIT2 v2.r837 and IMPUTE2 version 2.3.2 were used for data prephasing and genotype imputation. Data from the 1,000 Genomes phase 3 haplotypes were used as a reference panel. Imputed SNPs with info metric in IMPUTE2 below 0.5 were removed for this study.

After quality control and imputation, a total of 3,693,205 SNPs were used for this GWAS.

For each SNP that passed all the filtering criteria, we conducted a disease association study separately in men and women using a multiple linear regression model while controlling for age. Comparisons of continuous variables were performed using the Student *t* test, and categorical variables were compared using a χ^2 or the Fisher exact test. Analysis was performed using R statistical software package, version 3.1.1 (R Development Core Team; R Foundation for Statistical Computing, Vienna, Austria). SNPs with $P < 5.0 \times 10^{-8}$ were considered to be significant genome wide. To evaluate the combined effects of the significant SNPs on erosive esophagitis, we used the simple count method to calculate a genetic risk score in the discovery cohort. The results were considered statistically significant at a *P* value less than 0.05.

SNPs that had a *P* value less than 5.0×10^{-8} in the discovery set were re-evaluated for validation in the replication set. *P* values less than 0.05 were considered significant in the validation cohort.

Ethics statement

Approval was obtained from the Institutional Review Board (IRB) of the Seoul National University Hospital for the storage of biospecimens (IRB number 1103-127-357), which were used retrospectively. This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and its subsequent revisions and was approved by the board (IRB number 1610-102-801).

RESULTS

Baseline characteristics

A total of 4,242 participants were included in this investigation. Among the enrolled subjects, erosive esophagitis was diagnosed in 748 participants (17.6%): 586 (78.3%) in grade LA-A, 145 (19.4%) in grade LA-B, and 17 (2.3%) in grade LA-C or D. The diagnosis of erosive esophagitis was much higher in male subjects (22.3%) than in female subjects (9.8%). The characteristics of the discovery and validation cohorts according to sex are summarized in Table 1. Based on the accompanying endoscopic findings, hiatal hernia and BE were observed in 78 (10.4%) and 30 (4.0%) patients with erosive esophagitis, respectively (Table 2). A quantile–quantile plot is shown in Supplementary Digital Content 1 (see Figure 1, <http://links.lww.com/CTG/A220>). There was no factor that was significantly different between the cases and controls in both the discovery and replication cohorts.

GWAS for erosive esophagitis

The GWAS was conducted using 3,693,205 SNPs to identify genetic factors associated with erosive esophagitis. Because the demographic characteristics in erosive esophagitis varied according to sex, we performed genetic analysis separately in men and women. In the discovery set, we detected 6 genome-wide significant SNPs associated with erosive esophagitis in men: rs518309 ($P = 2.12 \times 10^{-8}$), rs654455 ($P = 2.12 \times 10^{-8}$), rs562589 ($P = 2.504 \times 10^{-8}$), rs594589 ($P = 2.786 \times 10^{-8}$), rs513126 ($P = 2.93 \times 10^{-8}$), and rs4445064 ($P = 5.864 \times 10^{-8}$). These SNPs are clustered near the *GRIK2* gene on chromosome 6. A regional plot for rs518309 is shown in Figure 1, and a Manhattan plot for the erosive esophagitis GWAS is shown in Supplementary Digital Content 2 (see Figure 2, <http://links.lww.com/CTG/A221>). We performed a validation test with a replication

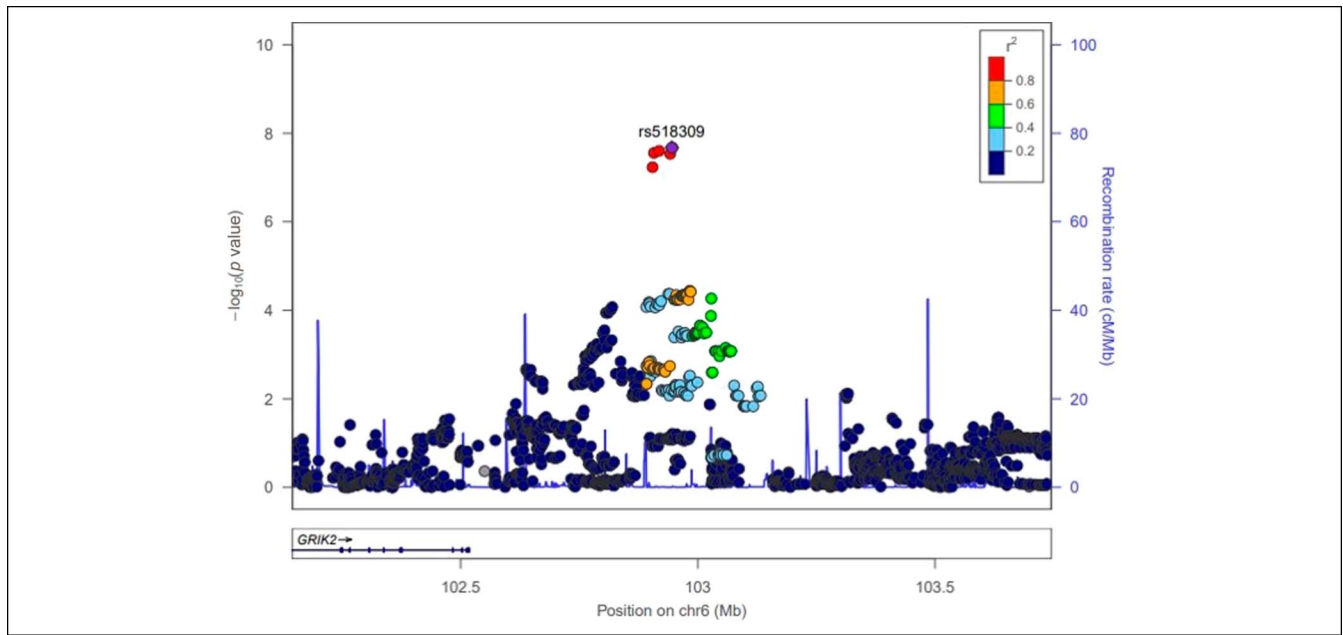


Figure 1. Regional plot for novel genome-wide significant single-nucleotide polymorphisms (SNPs) associated with erosive esophagitis in men. Associations of individual SNPs are plotted as $-\log_{10} P$ against the chromosomal position. The results of both genotyped and imputed SNPs are shown. Colors indicate the linkage disequilibrium strength between the most significant SNPs and the other SNPs assessed.

cohort, and all SNPs remained significant ($P < 0.05$) (Table 3). There were no significant SNPs found for female subjects.

Prediction of erosive esophagitis

We selected the 6 genome-wide significant SNPs for a prediction model of erosive esophagitis in male subjects. Erosive esophagitis was categorized into 4 groups (normal, LA-A, LA-B, and LA-C or D). In the case-control model, relative risk for erosive esophagitis was 1.08 (95% confidence interval [CI] = 1.05–1.12, $P < 0.0001$), with the control as a reference. According to the LA classification, the relative risks for severe reflux esophagitis were 1.08 (95% CI = 1.05–1.12, $P < 0.0001$) in LA-A, 1.09 (95% CI = 1.03–1.15, $P = 0.002$) in LA-B, and 1.16 (95% CI = 1.01–1.34, $P = 0.04$) in LA-C or D, as shown in Figure 2.

DISCUSSION

To the best of our knowledge, this is the first GWAS to have identified novel genome-wide significant SNPs associated with

erosive esophagitis proven by endoscopy. We detected 6 SNPs near the glutamate ionotropic receptor kainate type subunit 2 (*GRIK2*) gene on chromosome 6 as a novel marker for erosive esophagitis in male participants, and this variation significantly predicted erosive esophagitis through the genetic risk score.

Although many studies have made an effort to find genetic factors associated with GERD, they have failed to identify any genome-wide significant loci, in contrast to those found for BE and EAC (22). Both erosive esophagitis and NERD belong to GERD, which is characterized by heartburn and regurgitation, but it has recently been suggested that the underlying mechanism of development of erosive esophagitis is different from that of NERD (23). Although upper gastrointestinal endoscopies are the gold standard for diagnosis and classification of erosive esophagitis, they are not widely used in the Western countries because of cost-effectiveness and invasiveness. In Korea, there is a high prevalence of gastric cancer, and a screening upper

Table 3. Logistic regression analysis results of the genome-wide association study for erosive esophagitis in male subjects

| CHR | SNP | Position | Minor/major allele | Discovery set | | | Replication set | | |
|-----|-----------|-----------|--------------------|---------------|---------------------|-----------------------|-----------------|---------------------|----------------|
| | | | | MAF | OR (CI) | <i>P</i> value | MAF | OR (CI) | <i>P</i> value |
| 6 | rs518309 | 102945230 | C/T | 0.1499 | 1.703 (1.414–2.052) | 2.12×10^{-8} | 0.1295 | 1.623 (1.031–2.554) | 0.036 |
| 6 | rs654455 | 102946317 | A/G | 0.1499 | 1.703 (1.414–2.052) | 2.12×10^{-8} | 0.1295 | 1.623 (1.031–2.554) | 0.036 |
| 6 | rs562589 | 102918360 | C/G | 0.1505 | 1.698 (1.41–2.046) | 2.50×10^{-8} | 0.1298 | 1.617 (1.028–2.545) | 0.038 |
| 6 | rs594589 | 102907552 | A/G | 0.1507 | 1.695 (1.407–2.043) | 2.79×10^{-8} | 0.1298 | 1.617 (1.028–2.545) | 0.038 |
| 6 | rs513126 | 102941823 | T/C | 0.1504 | 1.694 (1.406–2.04) | 2.93×10^{-8} | 0.1295 | 1.623 (1.031–2.554) | 0.036 |
| 6 | rs4445064 | 102904579 | T/G | 0.1498 | 1.681 (1.393–2.027) | 5.86×10^{-8} | 0.1289 | 1.636 (1.039–2.576) | 0.034 |

CHR, chromosome; CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.

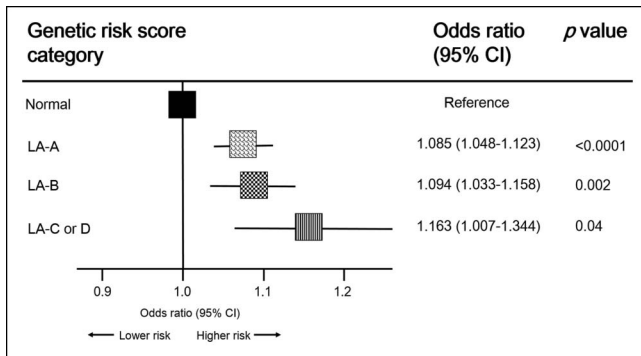


Figure 2. Prediction of erosive esophagitis risk in men using genetic scoring. Severity of reflux esophagitis is described by the LA classification: LA-A, LA-B, and LA-C or D. CI, confidence interval; LA, Los Angeles.

endoscopy is recommended for gastric cancer prevention. From the health checkup population, patients with erosive esophagitis found incidentally through upper endoscopy were included in this study. Thus, we could conduct the first GWAS for erosive esophagitis diagnosed by endoscopy, regardless of the GERD symptoms. In addition, we could evaluate the severity of erosive esophagitis and identify the relationship between the severity and genetic factors.

We found that genome-wide significant SNPs associated with erosive esophagitis only among male subjects. Similar to other studies, male subjects were found to have a 2-fold higher risk for erosive esophagitis compared with female subjects. Although being men is a powerful risk factor, erosive esophagitis is well known to be a multifactorial disease resulting from structural, environmental, hormonal, and genetic factors (2,10,11,24). Thus, we analyzed the baseline demographic data, including age, waist circumference, and smoking status. We found no significant differences in both our discovery and replication cohorts. Sex hormones could affect the prevalence and severity of GERD. In postmenopausal women, the prevalence of GERD rapidly increased, although it was lower than that in men in the reproductive period (25,26). In a recent study, rat models of erosive esophagitis showed male-predominant sex differences in esophageal damage through the protective effect of estrogen E2 receptor (27). This suggests that even if a female subject is at genetic risk for erosive esophagitis, she may also have a hormonal protective effect. This could help explain the significant genetic associations in male subjects only and elucidate the pathogenesis of erosive esophagitis.

The most genome-wide significant SNP (rs518309) is located 875 kb away from the *GRIK2* gene on chromosome 6q16.3. We found 6 genome-wide significant SNPs located in the topological domain of the chromosomal rearranged region of the *GRIK2* gene (3Disease Browser, <http://3dgb.cbi.pku.edu.cn/disease/>, <http://promoter.bx.psu.edu/hi-c/view.php>). The *GRIK2* gene encodes the glutamate ionotropic receptor kainate type subunit 2 (GRIK2), a member of the glutamate receptor family (28). Glutamate receptors play a role in synaptic excitation in neuronal cells, and these glutamatergic synapses are also found in the vago-vagal neural pathway (29–31). Transient lower esophageal sphincter (LES) relaxation is an important mechanism of GERD and is regulated by the vago-vagal neural pathway. In a recent study, the

metabotropic glutamate receptor 5 antagonist reduced gastroesophageal reflux episode by preventing transient LES relaxation and increasing LES pressure (32). We suggested that the *GRIK2* gene might be related to the pathogenesis of erosive esophagitis through transient LES relaxation via glutamatergic synapse.

In this cohort, hiatal hernia was more common in patients with erosive esophagitis (10.4%) than in normal controls (1.0%). Although the relationship between hiatal hernia and GERD has been discussed over the past decades, hiatal hernias are quite common among patients with GERD and may result in structural imperfections that affect the LES function (33). Gastric distension can potentially stimulate transient LES relaxation and induce anatomical vulnerabilities associated with hiatal hernia and predisposition to transient LES relaxation in patients with GERD (34). As mentioned above, erosive esophagitis is closely related to BE and EAC, but cases with BE were rare, and EAC was not observed in this study. This is likely because these 2 diseases are much rarer in Asians than in whites (35). Therefore, it is not presently possible to determine the relationship between the identified SNPs and these diseases.

Our study has several limitations. First, we selected the discovery and validation cohorts from the same population because this study was conducted by a single health screening center in Korea. As a result, the findings may not apply to individuals of other ethnic populations. Thus, a replication study should be conducted using a different population in the future. Second, we could not find significantly associated SNPs in female subjects. As mentioned previously, there may be a protective effect of estrogen affecting the development of erosive esophagitis in female subjects. In addition, the incidence of erosive esophagitis in women was low, and hence, a larger sample is needed for the study to have adequate statistical power. Third, these SNPs have not been previously reported in association with GERD, BE, and EAC. Because studies on these were mostly conducted in the Western populations, few have been conducted in Asian populations and so, these SNPs may not have been found to be significant because of ethnic differences. Finally, we were unable to examine GERD symptoms or a medication history because of the retrospective nature of the study.

In summary, we report the first GWAS of erosive esophagitis with 6 genome-wide significant SNPs in male subjects. These 6 SNPs near the *GRIK2* gene could help explain the pathogenesis of erosive esophagitis and contribute to the understanding of sex differences. Further genetic investigation of erosive esophagitis could allow for the prediction of high risk for erosive esophagitis and development of new treatment options.

CONFLICTS OF INTEREST

Guarantor of the article: Sung-Ae Jung, MD, PhD.

Specific author contributions: Eun Hyo Jin, MD, PhD, and Boram Park, MPH, contributed equally to this work. S.-A.J.: concept development and conducting the study. E.H.J.: drafting the manuscript and interpreting the data. B.P.: performing statistical analysis. Y.S.K.: writing review and editing. E.K.C.: collecting data and contributing methodology. S.H.C.: investigation and data curation. J.S.K.: conceptualization. All authors: approval of the final manuscript.

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Potential competing interests: None to report.

Study Highlights

WHAT IS KNOWN

- ✓ Erosive esophagitis is diagnosed by endoscopy and shows male-predominant sex difference.
- ✓ Genes affecting the susceptibility to erosive esophagitis have not yet been identified.

WHAT IS NEW HERE

- ✓ This GWAS stratified by sex investigated genetic factors associated with erosive esophagitis.
- ✓ Genome-wide significant SNPs associated with erosive esophagitis implicated *GRIK2* in men and predicted severity.

TRANSLATIONAL IMPACT

- ✓ These SNPs could help explain erosive esophagitis pathogenesis and inform the development of new therapies.

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