Ethnic variation in risk genotypes based on single nucleotide polymorphisms (SNPs) of the interferon-inducible transmembrane 3 (IFITM3) gene, a susceptibility factor for pandemic 2009 H1N1 influenza A virus

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Abstract
The interferon-inducible transmembrane 3 (IFITM3) protein is an effector of the host innate immune system that shows defensive activity against a wide range of viruses, including the influenza A virus. Previous studies have reported that three transcription-related regulatory single nucleotide polymorphisms (SNPs), rs12252, rs34481144, and rs6598045, showed potent associations with the severity of pandemic influenza A 2009 infection and susceptibility to this virus, respectively. However, the distribution of the risk genotypes of these three SNPs according to ethnic background has remained elusive. In this study, we compared the genotype and allele frequencies of the IFITM3 polymorphisms among several ethnic groups including American, African, European, South Asian, and East Asian using chi-square test. In addition, we analyzed the worldwide distribution of risk genotypes for pandemic influenza A 2009 virus infection. We found that the genotype and allele distributions of the rs12252, rs34481144, and rs6598045 SNPs were significantly different among several ethnic groups. In addition, the risk genotypes of the IFITM3 polymorphisms were also significantly different worldwide. To the best of our knowledge, this was the first simultaneous estimation of the risk genotypes of the IFITM3 gene with respect to pandemic influenza A 2009 virus infection.

Keywords IFITM3 · Interferon · SNP · rs12252 · rs34481144 · rs6598045 · Promoter region, pandemic influenza A 2009

Introduction
The interferon-inducible transmembrane 3 (IFITM3) protein is a potent antiviral effector encoded by the IFITM3 gene, which is classified as a small interferon-stimulated gene (Diamond and Farzan 2013; Feeley et al. 2011; Kim and Jeong 2017; Shi et al 2017; Zani and Yount 2018). The IFITM3 protein protects hosts from viral invasion by prohibiting hemifusion between host cells and various viruses, including the influenza A virus (Bailey et al. 2014; Brass et al. 2009; Li et al. 2013). The length of the human IFITM3 protein is relatively short (133 aa), and the IFITM3 protein contains an interspecific well-conserved CD225 domain (John et al. 2013). A previous study reported that ablation of the IFITM3 gene results in severe pneumonia in mildly influenza-infected mice and that elevated expression of the IFITM3 protein provides a dramatic increase in the survival rate of severely influenza-infected mice. These studies indicated that the expression level or integrity of IFITM3 protein can modulate the defensive capacity of the host immune system (Everitt et al. 2012). Thus, polymorphisms that can modulate the expression level or integrity of the IFITM3 protein are considered to be crucial factors in antiviral capacity. In addition, recent studies provide evidences that IFITM3 protein is also related to host adaptive immunity (Yánez et al. 2020). The expression of IFITM3 protein is regulated by T cell receptor (TCR) signal pathway and plays an essential role in T-helper Th1/Th2 differentiation (Bedford et al 2019; Wakim et al 2013; Yánez et al. 2019). Furthermore, IFITM3 protein is associated with antibody
response to flu vaccine and prevents cells from infection (Lei et al. 2020).

To date, nonsynonymous single nucleotide polymorphisms (SNPs) in the coding sequence (CDS) of the human IFITM3 protein that can induce amino acid sequence variation have not been identified. However, the rs12252 SNP-C allele-inducing splicing variant of the human IFITM3 protein denotes reduced antiviral capacity against pandemic influenza A 2009 virus infection (Everitt et al. 2012; Prabhu et al. 2018; Xuan et al. 2015; Zhang et al. 2013). In addition, recent studies have reported that two regulatory SNPs, rs34481144 and rs6598045, located in the promoter region regulate promoter activity by altering the binding affinity of the transcription factors CTCF and TFII-I, respectively. These two regulatory SNPs affected the transcriptional activity of the IFITM3 gene and showed an association with the severity and susceptibility of pandemic influenza A 2009 virus infection (Allen et al. 2017; Kim et al. 2020). The mechanism of action (MOA) and the association of three SNPs with pandemic influenza A 2009 virus infection were still unclear, and the difference of genotype and allele distributions of the IFITM3 polymorphisms according to ethnic background is also still elusive thus far.

In the present study, we compared the ethnic distribution of the genotype and allele frequencies of the rs12252, rs34481144, and rs6598045 SNPs of the IFITM3 gene involved in pandemic influenza A 2009 virus infection using data from the 1000 Genomes Project and a previously reported population. In addition, we investigated the difference of the risk genotype distributions of pandemic influenza A 2009 virus infection in each ethnic group.

### Materials and methods

#### Data collection

The genotype frequencies of rs12252, rs34481144, and rs6598045 SNPs of the IFITM3 gene in the Korean population were obtained from previous studies (Kim and Jeong 2017; Kim et al. 2020). The genotype distributions of these three SNPs were also collected from the 1000 Genomes Project.

#### Statistical analysis

Genotype and allele frequencies of the IFITM3 polymorphisms were compared by the chi-square test using SAS 9.4 Software (SAS Institute Inc., Cary, NC, USA).

### Results

#### Locations of the rs12252, rs34481144, and rs6598045 SNPs

The locations of the rs12252 SNP (c.42T>C), rs34481144 SNP (c.-23G>A), and rs6598045 SNP (c.-188T>C) are...
shown in Fig. 1. In brief, the rs12252 SNP was located in the CDS, the rs34481144 SNP was located in the 5′ untranslated region (UTR) of exon 1, and the rs6598045 SNP was located in the proximal promoter of the IFITM3 gene. The rs34481144 SNP is a transcription binding site of CTCF, and the rs6598045 SNP is a transcription binding site of TFII-I. Previous studies have reported that the binding ability of these transcription factors was modulated by the rs34481144 and rs6598045 SNPs (Allen et al. 2017; Kim et al. 2020).

Comparison of genotype and allele frequencies of the IFITM3 polymorphisms among several ethnic groups

We compared the genotype and allele frequencies of the IFITM3 polymorphisms, including rs12252, rs34481144, and rs6598045 SNPs among several ethnic groups. Detailed values are described in Tables 1 and 2. In brief, compared to rs12252, rs34481144, and rs6598045 SNPs of East Asian, those of the American, African, European, and South Asian showed significantly different distribution ($P < 0.05$). In Korean, compared to East Asian, except for rs34481144 SNP, the genotype and allele frequencies of rs12252 and rs6598045 SNPs showed significantly different distribution ($P < 0.05$).

Distributions of risk genotypes for pandemic influenza A 2009 virus infection in several ethnic groups

We also investigated risk genotypes with respect to pandemic influenza A 2009 virus infection. In brief, the risk genotype of the rs12252 SNP (CC genotype) was most frequently observed in East Asian populations (30%). However, European (0%), American (3%), and South Asian populations (3%) showed very low frequencies of the risk genotype of this SNP (Fig. 2). In addition, the risk genotype of the rs34481144 SNP (AA genotype) showed significantly different distributions among ethnic groups (Fig. 3). The risk genotype of rs34481144 was most frequently observed in European populations (22%). However, Korean, East Asian, and African populations did not carry the risk genotype of this SNP. Furthermore, the risk genotype of the rs6598045 SNP was most frequently observed in European populations (79%). However, compared to the European population, the African (46%) and South Asian populations (49%) showed lower frequencies of the risk genotype of this SNP (Fig. 4).

Table 1 Comparison of genotype frequencies of single nucleotide polymorphisms (SNPs) in the interferon-induced transmembrane protein gene (IFITM3) among several populations

| Polymorphisms | Populations | Total, n | Genotype frequencies | P value | Reference |
|---------------|-------------|----------|----------------------|---------|-----------|
| Rs12252 SNP   | American    | 235      | TT 101   CC 11      | $< 0.0001$ | 1000 genome project |
|               | African     | 363      | GG 252  GA 46      | $< 0.0001$ | 1000 genome project |
|               | European    | 462      | TT 41    CC 0       | $< 0.0001$ | 1000 genome project |
|               | South Asian | 360      | GG 114  GA 15      | $< 0.0001$ | 1000 genome project |
|               | East Asian  | 123      | TT 230  CC 151     | $< 0.0001$ | 1000 genome project |
|               | Korean      | 61       | GG 103  GA 41      | $0.0223$  | Kim and Jeong 2017; Kim et al 2020 |
| Rs34481144 SNP| American    | 213      | GG 106  GA 28      | $< 0.0001$ | 1000 genome project |
|               | African     | 605      | TT 55    CC 1       | $< 0.0001$ | 1000 genome project |
|               | European    | 148      | GG 245  GA 110     | $< 0.0001$ | 1000 genome project |
|               | South Asian | 305      | GG 166  GA 18      | $< 0.0001$ | 1000 genome project |
|               | East Asian  | 498      | TT 6     CC 0       | $< 0.0001$ | 1000 genome project |
|               | Korean      | 205      | TT 0     CC 0       | $0.1893$  | Kim and Jeong 2017; Kim et al 2020 |
| Rs6598045 SNP | American    | 199      | TT 142  TC 6       | $< 0.0001$ | 1000 genome project |
|               | African     | 305      | TT 317  TC 39      | $< 0.0001$ | 1000 genome project |
|               | European    | 400      | TT 99    CC 4       | $0.0089$  | 1000 genome project |
|               | South Asian | 239      | TT 229  TC 21      | $< 0.0001$ | 1000 genome project |
|               | East Asian  | 364      | TT 138  TC 2       | $< 0.0001$ | 1000 genome project |
|               | Korean      | 124      | TT 74    TC 7       | $0.0002$  | Kim and Jeong 2017; Kim et al 2020 |

Italicized texts indicate statistical significance ($P < 0.05$)
Table 2  Comparison of allele frequencies of single nucleotide polymorphisms (SNPs) in the interferon-induced transmembrane protein gene (*IFITM3*) among several populations

| Polymorphisms     | Populations  | Total, n | Allele frequencies | P value   | Reference                           |
|-------------------|--------------|----------|--------------------|-----------|-------------------------------------|
| Rs12252 SNP       | American     | 571      | T 123 C            | < 0.0001  | 1000 genome project                |
|                   | African      | 978      | T 344 C            | < 0.0001  | 1000 genome project                |
|                   | European     | 965      | T 41 C             | < 0.0001  | 1000 genome project                |
|                   | South Asian  | 834      | T 144 C            | < 0.0001  | 1000 genome project                |
|                   | East Asian   | 476      | T 532 -            |          | 1000 genome project                |
|                   | Korean       | 225      | T 185 -            | 0.0089    | Kim and Jeong 2017; Kim et al 2020 |
| Rs34481144 SNP    | American     | 532      | G 162 A            | < 0.0001  | 1000 genome project                |
|                   | African      | 1265     | G 57 A             | < 0.0001  | 1000 genome project                |
|                   | European     | 541      | G 465 A            | < 0.0001  | 1000 genome project                |
|                   | South Asian  | 776      | G 202 A            | < 0.0001  | 1000 genome project                |
|                   | East Asian   | 1002     | G 6 -              |          | 1000 genome project                |
|                   | Korean       | 410      | G 0 0              | 0.1903    | Kim and Jeong 2017; Kim et al 2020 |
| Rs6598045 SNP     | American     | 540      | T 154 C            | < 0.0001  | 1000 genome project                |
|                   | African      | 927      | T 395 C            | < 0.0001  | 1000 genome project                |
|                   | European     | 899      | T 107 C            | 0.0186    | 1000 genome project                |
|                   | South Asian  | 707      | T 271 C            | < 0.0001  | 1000 genome project                |
|                   | East Asian   | 866      | T 142 -            |          | 1000 genome project                |
|                   | Korean       | 322      | T 88 -             | 0.0006    | Kim and Jeong 2017; Kim et al 2020 |

Italicized texts indicate statistical significance (P < 0.05)

Fig. 2  Worldwide distribution of the risk genotype (CC genotype) of the rs12252 SNP of the *IFITM3* gene
**Fig. 3** Worldwide distribution of the risk genotype (AA genotype) of the rs34481144 SNP of the *IFITM3* gene

**Fig. 4** Worldwide distribution of the risk genotype (TT genotype) of the rs6598045 SNP of the *IFITM3* gene
Discussion

The *IFITM3* gene is classified as an interferon-stimulated gene (ISG) and a member of the IFITM protein family. The IFITM3 protein showed potent antiviral capacity against not only influenza A viruses but also Ebola virus (EBOV), Marburg virus (MARV), severe acute respiratory syndrome coronavirus (SARS-CoV), dengue virus (DEV), West Nile virus (WNV), and Zika virus (ZIKV) (Perreira et al. 2013; Zani and Yount 2018). Since the IFITM3 protein plays a pivotal role in blocking the invasion of several viruses, polymorphism studies that affect protein integrity or expression levels have received attention. Previous case–control studies and meta-analyses have confirmed the association of the rs12252 SNP with the severity of pandemic influenza A 2009 virus infection (Chen et al. 2018; Prabhu et al. 2018; Xuan et al. 2015; Yang et al. 2015). However, a study in a Korean population did not confirm the correlation of the disease severity of pandemic influenza A 2009 virus infection with the rs12252 SNP of the *IFITM3* gene (Kim and Jeong 2017).

In addition, the European population did not carry the risk genotype of the rs12252 SNP. Since these studies did not include correlation analysis of the worldwide distribution of pandemic influenza A 2009 virus-infected patients, a follow-up study suggested the rs34481144 SNP as a novel risk factor for pandemic influenza A 2009 virus infection. The rs34481144 SNP is located in a CpG island, and the A allele of the rs34481144 SNP disrupts the binding site of CTCF. In addition, the rs34481144 SNP acts as not only a proximal promoter of the *IFITM3* gene but also a distal promoter of adjacent genes, including *IFITM1, IFITM2,* and *B4GALNT4.* These results indicate that the rs34481144 SNP is an important site and regulates cascade-like *IFITM3*-related immune signaling. However, the Korean population did not harbor the rs34481144 SNP (Allen et al. 2017). In a recent study, the genotype of the rs6598045 SNP, which is located in the proximal promoter of the *IFITM3* gene, modulated the transcriptional activity of the *IFITM3* gene and was significantly associated with susceptibility to pandemic influenza A 2009 virus infection in the Korean population (Kim et al. 2020). Although the rs12252, rs34481144, and rs6598045 SNPs are related to the severity of pandemic influenza A 2009 infection or susceptibility to this virus, the ethnic distribution of the risk genotypes of these three SNPs has not been summarized thus far.

In the present study, we found significant difference of the genotype and allele frequencies of the *IFITM3* polymorphisms among several ethnic groups. In addition, we summarized ethnic variations in the risk genotypes of the rs12252, rs34481144, and rs6598045 SNPs. Interestingly, the European, American, and South Asian populations showed very low frequencies of the risk genotype of the rs12252 SNP (Fig. 2). In addition, the Korean, East Asian, and African populations did not carry the risk genotype of the rs34481144 SNP (Fig. 3). Furthermore, the risk genotype of the rs6598045 SNP showed variation among ethnic groups (Fig. 4). Since no single SNP could fully explain the severity or susceptibility of pandemic influenza A 2009 virus infection, evaluation of susceptibility based on haplotypes of the *IFITM3* polymorphisms is highly desirable in the future. In addition, a recent study has been reported that rs12252 SNP is associated with severity of coronavirus disease 2019 (COVID-19) (Zhang et al. 2020). Thus, evaluation on an association between *IFITM3* SNPs and susceptibility or severity of COVID-19 is needed in the future.

Since the IFITM3 protein has a highly homologous CD225 domain and is conserved among several species, it is necessary to investigate it in several viral host animals. In primate genome, IFITM3 protein is the most ancient antiviral protein of the IFITM locus and has undergone repeated duplication in each independent lineage. In addition, some *IFITM3* genes in non-human primates have amino-terminal variations that alter protein localization and function (Compton et al. 2016). A recent study reported a difference in the expression level of IFITM3 protein between avian influenza-susceptible animals and avian influenza-resistant animals (Smith et al. 2015). In addition, a difference in the genetic distribution of polymorphisms of the chicken *IFITM3* gene was also identified between layers and broilers, which are bred for eggs and meats, respectively (Kim et al. 2019, 2020). Furthermore, since pig IFITM3 protein showed a defensive capacity against classical swine fever virus (CSFV), African swine fever virus (ASFV), and foot and mouth disease virus (FMDV), further investigation of the polymorphisms of animal *IFITM3* genes is needed in the future (Li et al. 2019; Munoz-Moreno et al. 2016; Xu et al. 2014).

In conclusion, we investigated the ethnic distribution of the risk genotypes of the rs12252, rs34481144, and rs6598045 SNPs with respect to pandemic influenza A 2009 virus infection. We observed significant differences in these three SNPs among the ethnic groups. In addition, we investigated the distribution of risk genotypes for pandemic influenza A 2009 virus infection. To the best of our knowledge, this is the first report of simultaneous evaluation of risk genotypes of the *IFITM3* gene with respect to pandemic influenza A 2009 virus infection.

Author contributions YCK and BHJ conceived and designed the experiment. YCK performed the experiments. YCK and BHJ analyzed the data. YCK and BHJ wrote the paper. All authors read and approved the final manuscript.
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Data availability All data generated or analyzed during this study are available from the corresponding author on reasonable request.

Compliance with ethical standards Conflict of interest The authors declare that they have no conflict of interest.

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