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IFITM3 rs12252 T>C polymorphism is associated with the risk of severe influenza: a meta-analysis

Y. XUAN[1], L. N. WANG[1,2], W. LI[3], H. R. ZI[1], Y. GUO[1], W. J. YAN[1], X. B. CHEN[1] AND P. M. WEI[1]*

[1] *Key Laboratory of Environmental Medicine Engineering, Ministry of Education, Department of Epidemiology & Biostatistics, School of Public Health, Southeast University, Nanjing, China*
[2] *School of Biological Science and Medical Engineering, Southeast University, Nanjing, China*
[3] *Department of Infectious Disease Prevention and School Health, Nanjing Municipal Center for Disease Control and Prevention, Jiangsu, China*

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SUMMARY

The interferon-inducible transmembrane protein 3 (*IFITM3*), as one of the key genes involved in the interferon pathway, is critical for defending the host against influenza virus, and the rs12252 T>C variant in *IFITM3* might be associated with susceptibility to severe influenza. Owing to contradictory and inconclusive results, we performed a meta-analysis to assess the association between rs12252 T>C polymorphism and severe influenza risk. A comprehensive literature search up to 1 August 2014 was conducted in EMBASE, Pubmed, Web of Science, VIP, Wanfang and CNKI databases. Four eligible studies with a total of 445 influenza patients and 3396 controls were included in this meta-analysis. Overall, our results demonstrated a significant association between the *IFITM3* rs12252 T>C polymorphism and influenza risk [C *vs.* T: odds ratio (OR) 1·68, 95% confidence interval (CI) 1·32–2·13; CC *vs.* CT+TT: OR 2·38, 95% CI 1·52–3·73; CC+CT *vs.* TT: OR 1·62, 95% CI 1·18–2·22]. Stratification by ethnicity indicated that the variant C allele was associated with an 88% increased risk of influenza in Asians (C *vs.* T: OR 1·88, 95% CI 1·34–2·62). Moreover, subjects carrying the variant C allele had an increased risk of developing severe illness upon influenza infection (C *vs.* T: OR 2·70, 95% CI 1·86–3·94). However, no significant association was observed in patients with mild infection (C *vs.* T: OR 1·26, 95% CI 0·93–1·71). Our meta-analysis suggests that *IFITM3* rs12252 T>C polymorphism is significantly associated with increased risk of severe influenza but not with the chance of initial virus infection.

Key words: *IFITM3*, influenza, meta-analysis, polymorphism.

INTRODUCTION

Influenza continues to be a major cause of morbidity and mortality worldwide and places a considerable socioeconomic burden on humans and society. Seasonal influenza outbreaks cause as many as 5 million cases of severe illness in humans and half a million deaths worldwide annually [1]. In addition to seasonal influenza, a new influenza strain resulting from antigenic shift or reassortment which demonstrates human-to-human transmission can compromise vaccine and immune effectiveness, with novel epidemics or global pandemics resulting in millions of fatalities [2]. Patients with influenza virus infection,

* Author for correspondence: Professor P. M. Wei, Key Laboratory of Environmental Medicine Engineering, Ministry of Education, Department of Epidemiology & Biostatistics, School of Public Health, Southeast University, 87 Ding Jiaqiao Road, Nanjing 210009, China.
(Email: mpw1963@126.com)

including the pandemic influenza A(H1N1)pdm/09 virus (pdmH1N1/09), first detected in 2009, and the avian influenza A(H7N9) virus (H7N9), first reported in 2013, show great variability in the severity of disease, ranging from mild to severe. This variability could result from three main factors: the intrinsic virulence of the virus, acquired host factors (e.g. immunity) and inherent host susceptibility [3–5]. Viral genetic factors involved in influenza severity and host immunity have been thoroughly studied, nevertheless, host genetic factors are still not fully understood. Population-based studies have shown that the host genetic background may play an important role in the aetiology of influenza and even in the severity of disease [6, 7]. Although there were many unrecognized acquired susceptibility factors that may play a role in susceptibility to severe disease in apparently 'healthy' individuals, such as nutritional imbalances, the composition of the upper respiratory tract microbiome or co-infection, host genetics may also be important and should be considered. Up to now, evidence for an effect of host genetics on influenza severity in humans has only been demonstrated for the interferon-inducible transmembrane protein 3 (*IFITM3*) gene [3, 5].

IFITM proteins 1, 2, and 3 were found to inhibit the early replication of multiple subtypes (H1, H3, H5, H7) of influenza A virus and *IFITM3* was the crucial restriction factor [8]. *IFITM3* is one of the interferon-stimulated genes and plays a role in antivirus responses by inducing the expression of IFITM3 protein. Using a knockout mouse model and A549 lung carcinoma cell lines, *IFITM3* expression was identified as a critical barrier to influenza A virus infection *in vivo* and *in vitro*. The human *IFITM3* gene is located on chromosome 11, has two exons and encodes two splice variants which differ by a length of 21 amino acids in the first amino terminus. The minor rs12252-C (major T allele) alters a splice acceptor site and may be related to the *IFITM3* splice variant, which encode a truncated protein lacking the first 21 amino acids, leading to reduced ability of *IFITM3* to act as an antiviral agent [9, 10]. In 2012, Everitt *et al*. found that patients who developed severe illness following pdmH1N1/09 infection were more likely to carry the minor rs12252-C allele [10]. However, Mills *et al.* reported that rs12252 CC homozygotes are associated with susceptibility to mild but not severe influenza infection [11]. The discrepancy between these results may be due to comparatively small sample sizes, inadequate statistical power and the ethnic diversity of the population. To become better acquainted with its possible influence on influenza infection, we performed this meta-analysis. To the best of our knowledge, this is the first meta-analysis to evaluate the association between the rs12252 polymorphism and severe influenza.

MATERIALS AND METHODS

The meta-analysis was designed and performed following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [12].

Publication search strategy

We searched the following databases from their establishment until 1 August 2014: Pubmed, EMBASE, Web of Science, Wanfang and CNKI (China National Knowledge Infrastructure). The following key words and medical subject headings were employed: (polymorphism OR genotype OR genetic variant OR allele OR SNP) AND (interferon-inducible transmembrane protein 3 or *IFITM3*) AND (influenza or flu). Additional studies were identified by a manual search of bibliographies of original or review articles regarding this topic. There was no limitation on the language of publication.

Inclusion criteria and exclusion criteria

Studies were included which met the following criteria: (1) evaluated the association between the *IFITM3* rs12252 polymorphisms and influenza risk, (2) were designed in a clinical cohort or case-control study, and (3) contained sufficient data for estimating odds ratio (ORs) with their corresponding 95% confidence intervals (CIs). Studies were excluded when they were: (1) review articles, comments and published abstracts from meetings, (2) not related to the *IFITM3* rs12252 polymorphism and influenza, and (3) not human subjects. When there were multiple studies with the same or overlapping data, only the most recent study with the largest sample sets was selected for this meta-analysis. Study selection was achieved by two investigators independently, according to the inclusion and exclusion criteria, by screening the title, abstract and full text. Any disagreement was solved by discussion. A flow chart for the process of study retrieval and exclusion is shown in Fig. 1.

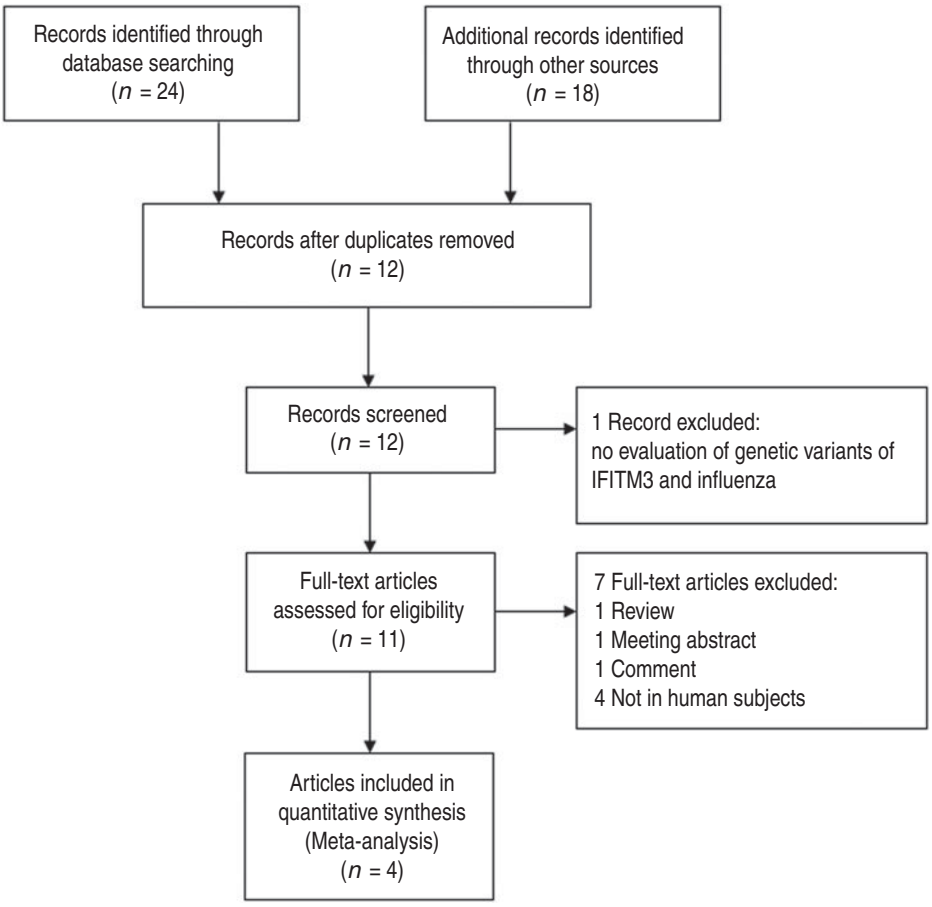

Fig. 1. Flow diagram of study selection. Studies were selected based on inclusion and exclusion criteria.

Data extraction and quality assessment

Data were extracted from all selected studies by two independent investigators (Y.X. and L.N.W.). The following data were collected from each selected study: the first author's name, publication year, country of origin, ethnicity of study population, influenza type, sex and age of population, number of eligible cases and controls, genotyping method, the frequencies of the alleles and the genotypic distributions for both the cases and controls and the Hardy–Weinberg equilibrium (HWE) in controls (*P* value). Different ethnicities were classified as Caucasian and Asian. If the distribution of alleles was not given, it was calculated by genotype distribution. Authors of the selected studies were contacted via email if further study information was needed. Inter-researcher conflict was resolved by discussion. If disagreement still persisted, a third investigator would participate in order to settle the dispute (P.M.W.).

The methodological quality of the included studies was evaluated independently by the same two investigators according to the Newcastle–Ottawa Scale (NOS) [13]. The NOS criteria consists of three aspects: selection, comparability and exposure. Scores ranged from 0 stars (worst) to 9 stars (best) and a score $\geqslant 7$ indicated that a study was of high quality. Dissent was settled as described above.

Statistical analysis

Initially, we assessed HWE for each study by using χ^2 test in the controls groups, and $P < 0{\cdot}05$ was considered a significant departure from HWE. Under the allelic model (C *vs.* T), dominant model (CC+CT *vs.* TT), recessive model (CC *vs.* CT+TT), we evaluated the strength of associations between *IFITM3* single nucleotide polymorphism (SNP) and influenza by calculating a pooled OR and 95% CI. The statistical significance of OR was determined by *Z* test, and $P < 0{\cdot}05$ was considered statistically significant.

Data heterogeneity was evaluated using Cochran's *Q* statistic and the I^2 test [14, 15]. $P < 0{\cdot}1$ and $I^2 >$

Table 1. *Main characteristics of the studies included in the meta-analysis*

Study, first-named author	Country	Ethnicity	Influenza type	Sex (M/F)	Age, yr	Underlying medical conditions	Samples of severe infection	Samples of mild infection	Samples of controls	Genotyping method	NOS score
Everitt, 2012 [10]	UK	Caucasian	pdmH1N1/09, seasonal influenza virus	29/24	37 (2–62)	6 (12%)	53	–	379*	nested PCR	7
Mills, 2014 [11]	Mixed	Caucasian	H1N1	–	Adult	–	34	259	2623†	PCR	8
Wang, 2014 [4]	China	Asian	H7N9	12/4	68·25 ± 11·16	11 (69%)	16	–	197*	PCR	7
Zhang, 2013 [3]	China	Asian	pdmH1N1/09	50/33	24·55 ± 13·92	–	32	51	197*	PCR	7

pdmH1N1/09, Influenza A(H1N1)pdm09; H1N1, influenza A(H1N1); H7N9, avian influenza A(H7N9); M, male; F, female; PCR, polymerase chain reaction; NOS, Newcastle–Ottawa scale.
* Data obtained from the 1000 Genome Project.
† Genomics to combat Resistance against Antibiotics in Community acquired LRTI in Europe (GRACE) study controls.

50% indicated heterogeneity across studies, in this case a random-effects model was used, otherwise a fixed-effects model was applied. Either a fixed-effects or random-effects model was applied to pool the effect sizes according to heterogeneity [16].

The studies whose controls were in agreement with HWE were used to perform a supplementary meta-analysis. Because the potential causes of heterogeneity in studies were genetic differences between the races, we conducted stratification analyses on ethnicity (Asian and Caucasian). Additionally, a subgroup analysis of patients with varying degrees of symptoms (severe infection and mild infection) was also performed. The stability of the summary risk estimate was assessed using a sensitivity analysis in which each study was individually removed and the OR was recomputed.

Begg's funnel plot and Egger's regression test were used to search for publication bias [17, 18]. The fail-safe number (N_{fs}) set at a significance of 0·05 was also computed to detect publication bias, according to the formula $N_{fs0\cdot05} = (\sum Z/1\cdot64)^2 - k$, where k equals the number of studies included. All statistical analyses were performed using Stata v. 12.0 software (StataCorp, USA).

RESULTS

The main characteristics of the selected studies

A total of four studies that meet the inclusion criteria were included in this meta-analysis. The general characteristics of all included studies are summarized and presented in Table 1. The four selected articles were clinical cohort studies and were published from 2012 to 2014, including a total of 445 influenza cases and 3396 healthy controls. Two types of patients with varying degrees of symptoms were addressed: severe infection and mild infection. Of the four articles, all investigated the association between the *IFITM3* rs12252 polymorphism and patients with severe infection [3, 4, 10, 11], and two also investigated the relationship with mild infection [3, 11]. Two of the studies were conducted in Asian populations containing a total of 99 cases and 394 controls, while two were in European populations with 346 patients and 3002 controls. The NOS scores of all included studies ranged from 7 to 8, indicating the methodological quality was generally good.

The genotype and allele distributions of rs12252 T>C polymorphisms are shown in Table 2. The

Table 2. *Distribution of* IFITM3 *rs12252 T>C polymorphism in studies included in the meta-analysis*

	Case					Control						HWE	
Study, first-named author	CC	CT	TT	C	T	CC	CT	TT	C	T	MAF	χ^2	*P*
Everitt, 2012 [10]	3	4	46	10	96	0	26	353	26	732	0·034	0·478	0·4893
Mills, 2014 [11]	2	25	266	29	557	4	202	2417	210	5036	0·04	0·011	0·9178
Wang, 2014 [4]	6	7	3	19	13	52	72	73	176	218	0·447	13·382	0·0003
Zhang, 2013 [3]	35	39	9	109	57	50	98	49	198	196	0·503	0·005	0·9435

MAF, Minor allele frequency; HWE, Hardy–Weinberg equilibrium.

genotype distributions of the control groups in the studies were in agreement with HWE, except in one study [4]. The minor allelic frequency (MAF) of the controls in different ethnicities varied greatly, ranging from 0·034 in Europeans to 0·503 in Chinese.

Association between *IFITM3* rs12252 T>C polymorphism and influenza susceptibility

Four studies (445 cases, 3396 controls) have assessed the relationship between the rs12252 polymorphism and influenza risk. The main results of this relationship are presented in Figure 2. Since no significant heterogeneity was detected by the *Q* test and I^2 statistic, the fixed-effects model was used for all the genetic models. Compared with the wild allele T, the *IFITM3* rs12252-C allele was associated with a 68% increased risk of influenza, and the significant effects were also shown in the dominant and recessive models (C *vs.* T: OR 1·68, 95% CI 1·32–2·13, $P < 0·001$; CC *vs.* CT +TT: OR 2·38, 95% CI 1·52–3·73, $P < 0·001$; CC+CT *vs.* TT: OR 1·62, 95% CI 1·18–2·22, $P = 0·003$).

The analysis stratified by ethnicity showed that Caucasians with the rs12252 CC genotype had a higher susceptibility to influenza than those with rs12252 CT/TT genotypes in the fixed-effects model (CC *vs.* CT+TT: OR 10·62, 95% CI 2·99-37·74, $P < 0·001$); but no significant association was found for the allele model and dominant model (C *vs.* T: OR 1·80, 95% CI 0·78–4·12, $P = 0·167$; CC+CT *vs.* TT: OR 1·30, 95% CI 0·89–1·90, $P = 0·170$). While for Asians, the significant association between the *IFITM3* rs12252 polymorphism and susceptibility to influenza was found in three different genetic models (C *vs.* T: OR 1·88, 95% CI 1·34–2·62, $P < 0·001$; CC *vs.* CT+TT: OR 2·04, 95% CI 1·26-3·03, $P = 0·004$; CC+CT *vs.* TT: OR 2·68, 95% CI 1·39–5·16, $P = 0·003$). In addition, for patients with severe infection, a significantly increased risk of severe influenza was identified in genetic models (C *vs.* T: OR 2·70, 95% CI 1·86–3·94, $P < 0·001$; CC *vs.* CT+TT: OR 5·28, 95% CI 1·64–16·95, $P = 0·005$; CC+CT *vs.* TT: OR 2·35, 95% CI 1·36–4·06, $P = 0·002$). For patients with mild infection, no statistically significant association was observed for any genetic model (C *vs.* T: OR 1·26, 95% CI 0·93–1·71, $P = 0·134$; CC *vs.* CT+TT: OR 1·87, 95% CI 0·39–8·90, $P = 0·430$; CC +CT *vs* TT: OR 1·37, 95% CI 0·94–2·02, $P = 0·104$) (Table 3). However, only two studies were available for pooled estimation of the association, hence this result should be interpreted with caution.

Sensitivity analysis and publication bias

Sensitivity analyses were conducted to evaluate the influence of single studies on the pooled results by omitting individual studies in turn. After excluding the study deviating from HWE, we arrived at almost the same results (the relative results are not given in the text).

Potential publication bias was examined by Begg's funnel plot and Egger's test. The shape of the funnel plots showed no evidence of obvious asymmetry in any genetic models (Fig. 3). Egger's test also indicated a lack of publication bias for all genetic models (C *vs.* T: $t = 0·96$, $P = 0·440$; CC+CT *vs.* TT: $t = 2·37$, $P = 0·141$; CC *vs.* CT+TT: $t = 1·81$, $P = 0·212$). The $N_{fs0·05}$ of this study was 26, which is greater than the number of studies included in our meta-analysis, implying a low probability of publication bias.

DISCUSSION

The roles of genetic polymorphisms in susceptibility to influenza are much less well studied but are good candidates for investigation. A number of candidate genes have been proposed for susceptibility to severe influenza, such as the *IFITM3* and *TNF* genes. However, to the best of our knowledge, evidence of an association in humans has only been shown for

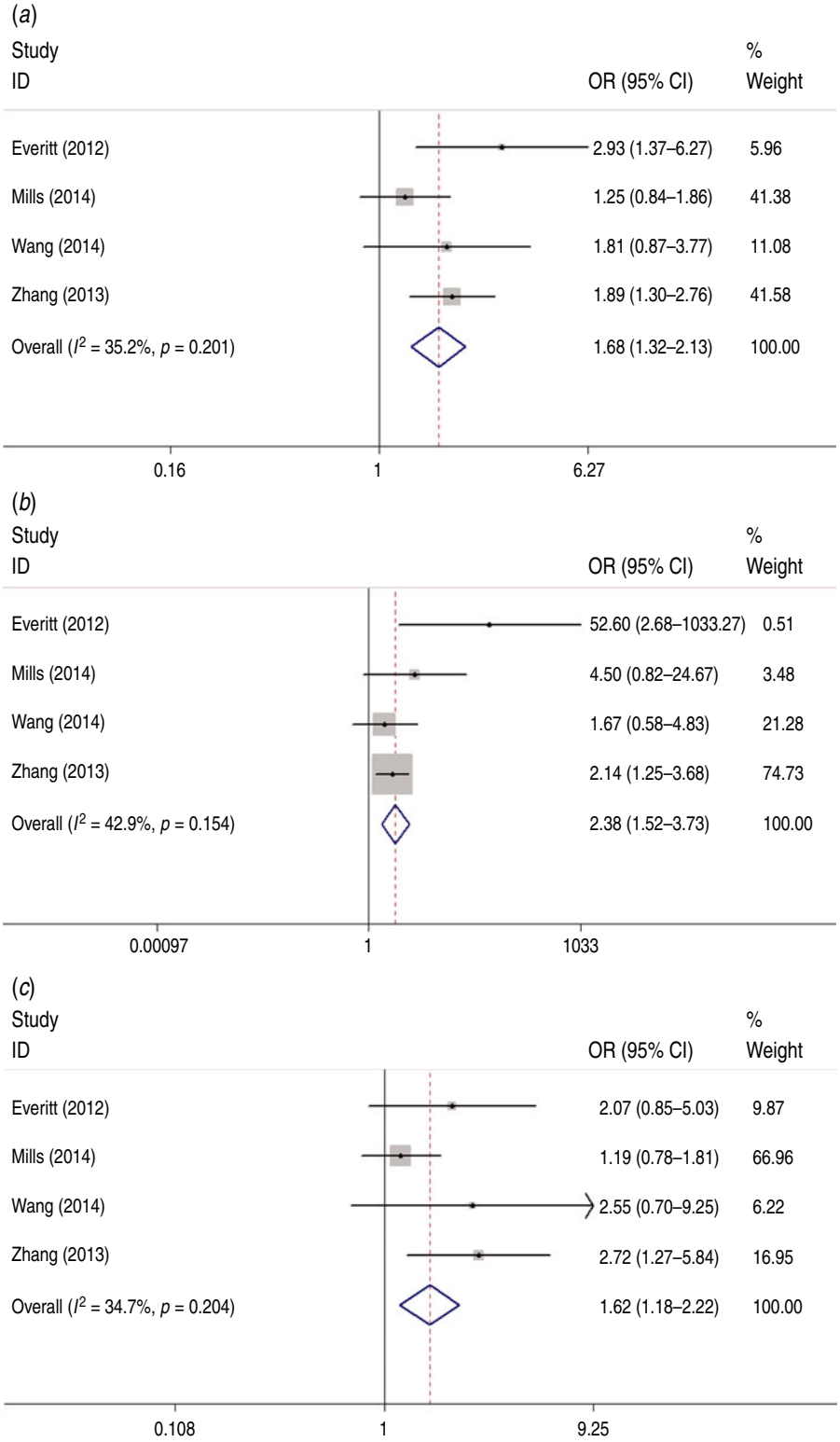

Fig. 2. The odds ratios of the *IFITM3* rs12252 polymorphism and influenza risk in different genetic models. (*a*) C *vs*. T; (*b*) CC *vs*. CT+TT; (*c*) CC+CT *vs*. TT. The fixed-effects model was used to pool the effect sizes for all the genetic models.

Table 3. *Summary odds ratios of the association between* IFITM3 *rs12252 polymorphism and influenza risk by ethnicity and symptom severity*

		C *vs.* T			CC+CT *vs.* TT			CC *vs.* CT+TT		
Variables	*N**	OR (95% CI)	$P^{\dagger}$	I^2 (%)	OR (95% CI)	$P^{\dagger}$	I^2 (%)	OR (95% CI)	$P^{\dagger}$	I^2 (%)
Ethnicity										
Caucasian	2	1·80 (0·78–4·12)	0·050	73·9	1·30 (0·89–1·90)	0·272	17·2	10·62 (2·99–37·74)	0·149	52·1
Asian	2	1·88 (1·34–2·62)	0·915	0·0	2·68 (1·39–5·16)	0·932	0·0	2·04 (1·26–3·30)	0·683	0·0
Symptom severity										
Severe infection	4	2·70 (1·86–3·94)	0·149	43·8	2·35 (1·36–4·06)	0·471	0·0	5·28 (1·64–16·95)	0·075	56·5
Mild infection	2	1·87 (0·39–8·90)	0·082	66·9	1·37 (0·94–2·02)	0·262	20·6	1·26 (0·93–1·71)	0·972	0·0

OR, Odds ratio; CI, confidence interval.
* Number of comparisons.
† *P* value for heterogeneity.

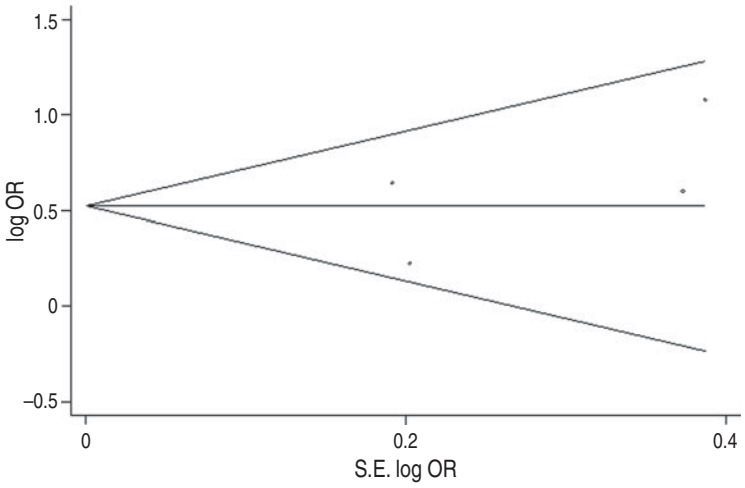

Fig. 3. Begg's funnel plot for publication bias analysis for *IFITM3* polymorphism rs12252 (C *vs.* T). Begg's funnel plot was used to detect potential publication bias.

the *IFITM3* gene. *IFITM3* is one of the interferon-stimulated genes which can induce the expression of IFITM3 proteins, and then confer basal resistance to the influenza virus. Rs12252 T>C polymorphism in the human *IFITM3* gene has been identified in the translated region of the human *IFITM3* coding sequence. In 2012, Everitt and colleagues reported that an association exists between the *IFITM3* rs12252 polymorphism and susceptibility to severe influenza A(H1N1) virus (H1N1) or seasonal influenza infection, and found that a statistically significant number of patients hospitalized with influenza virus infection showed an enrichment of a minor *IFITM3* allele (SNP rs12252-C), and that *IFITM3* CC homozygotes reduced influenza virus inhibition *in vitro* [10]. But Mills *et al.* did not find this SNP to be associated with susceptibility to severe H1N1 infection in British patients, whereas a most significant association with susceptibility to mild influenza infection was identified [11]. Estimation of the relationship might be based on small sample size, and the conflicting results are unsurprising. Hence, the meta-analysis is imperative to guarantee sufficient statistical power.

In this meta-analysis, we performed a systematic literature search in different databases and included four independent studies with a total of 445 influenza

cases and 3396 healthy controls. Overall, a statistically significant association between *IFITM3* rs12252 polymorphisms and susceptibility to influenza was identified in different genetic models. It is noteworthy that humans who carry the variant C allele were associated with a 68% increased risk of influenza compared to subjects who carry the T allele, and a significantly increased risk of influenza was also found in recessive and dominant models. The phenomenon may be related to the rare SNP rs12252-C alleles which truncate the protein, leading to reduced inhibition of influenza virus replication.

In this study, we performed a subgroup analysis by ethnicity. In Asians, pooled estimates showed that the rs12252-C variant allele might be a risk factor for influenza virus infection in all genetic models. In Caucasians, individuals with homozygous CC had a 9·62-fold increased risk for influenza than carriers of the CT/TT genotype. Although the risk in Caucasian subjects with the C allele was ~1·8 times as great as for individuals with the T allele, the result was not significant ($P = 0{\cdot}167$). The differences may be explained by genetic diversity and the MAF varied greatly by ethnic background. Meanwhile, gene–gene and gene–environment interactions may also lead to the complexity of the genetic effect. Previous studies report that the rs12252 T/C polymorphism was associated with disease severity rather than the increasing chance of initial virus infection [3]. To derive a more precise assessment of the relationship, we conducted a stratified analysis by classifying study subjects as severe infection cases and mild infection cases. With regard to patients with severe infection, a significantly increased risk for developing severe illness following influenza virus infection was identified in all genetic models. Together, these results suggest that the variant C allele is associated with severity of illness but does not lead to increased susceptibility to infection.

It is well known that influenza severity was determined by a complex interaction between influenza viruses and the co-existing medical conditions of the body [5, 19]. For example, during the period of the H7N9 outbreak or H1N1 influenza pandemic in 2009, individuals with co-existing conditions such as coronary artery disease, diabetes, chronic obstructive pulmonary disease, or hypertension, and with H7N9 virus or pdm09H1N1 virus infection, were more vulnerable to suffer a less favourable or fatal clinical outcome [4, 19, 20]. Moreover, the subtype of influenza virus was also a key point that determines the severity of disease in humans. Multiple studies have found that individuals with pdmH1N1/09 infection might suffer a relatively mild disease course similar to other human influenza A infections [3], whereas infection of patients with the highly pathogenic H7N9 strain were more likely to have a poor or fatal outcomes [4]. The pdmH1N1/09 strain generally lacks mutations that have previously been shown to confer increased virulence, pathogenicity or transmissibility in other influenza A viruses [20]. However, it is noteworthy that most H7N9 isolates possess the Q226L and G186 V substitution in the haemagglutinin (*HA*) gene, E627 K substitution in the *PB2* gene and a five amino-acid deletion in the stalk region of neuraminidase (NA), which might be associated with transmission, mammalian adaptation or virulence of H7N9 viruses [21–24].

More recently, the C allele and CC genotype for rs12252 have been identified that appear to be associated with the development of coronary artery lesions in Kawasaki disease (KD) patients. Since the rs12252-C allele of *IFITM3* truncates the protein, resulting in reduced restriction of viral emergence from the endosomal pathway, it could be assumed that enveloped viruses may play a vital role in the aetiology of KD coronary artery lesions. In addition, KD patients with *IFITM3* dysfunction are predisposed to enhanced inflammatory responses and tissue damage. These findings contribute to a better understanding of the functional role of rs12252 polymorphism in KD coronary artery lesions [25].

Heterogeneity is a potential challenge that may influence the explanation of the results. In our meta-analysis, no obvious heterogeneity was detected in any genetic models. However, in analysis stratified by ethnicity, significant heterogeneity was found in Caucasians in the allele model. The small sample size may contribute to the heterogeneity. Moreover, when stratified by varying degrees of symptoms, heterogeneity was found in the recessive model in both subgroups and the analyses were performed using the random-effects model. Additionally, our sensitivity analysis, excluding one study deviating from HWE, yielded almost similar results, indicating that our results were stable and reliable. No apparent publication bias was identified, strengthening this conclusion.

Although this is the first study, to the best of our knowledge, to describe the *IFITM3* rs12252 T>C polymorphisms and susceptibility to influenza infection with a relatively large study population size with high statistical power (if we set $\alpha = 0{\cdot}05$, based on the dataset for rs12252 CC, we have an 80%

power to detect an OR of 1·945) some limitations should be taken into consideration. First, the number of published studies for the *IFITM3* rs12252 polymorphisms selected in our meta-analysis was limited. It is possible that several unpublished articles with negative findings are missing. Hence, more studies were requested to verify the association. Second, one study was not in accord with HWE expectations. However, when the analysis was restricted to the studies in HWE, the pooled ORs for the *IFITM3* rs12252 polymorphisms and influenza risk remained significant in all genetic models. Third, our outcome was based on unadjusted estimates due to the lack of original data. Thus, if individual information (such as gender or age) had been available to allow for adjustment, a more precise analysis could have been performed. Fourth, only four studies were included, so we could not perform the stratified analysis by subtype of influenza virus because of reduced power. Finally, in several of the included studies, the data for healthy controls was obtained from the 1000 Genomes Project [3, 4, 10]. Therefore, the controls may not be representative of the underlying source populations.

In conclusion, this meta-analysis suggests that the *IFITM3* rs12252 CC variant genotype is significantly associated with an increased risk of infection to severe influenza but not with the increasing susceptibility to initial virus infection. Thus, the *IFITM3* rs12252 genetic variant might be a potential biomarker for the early diagnosis of severe influenza. However, due to the above-mentioned limitations, future well-designed studies with larger sample sizes are needed to validate the association.

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DECLARATION OF INTEREST

None.

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