BRIEF REPORT

IFITM3 and Susceptibility to Respiratory Viral Infections in the Community

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Interferon-inducible transmembrane proteins 1, 2, and 3 (IFITM 1,2, and 3) are viral restriction factors that mediate cellular resistance to several viruses. We have genotyped a possible splice-site altering single-nucleotide polymorphism (rs12252) in the *IFITM3* gene in 34 patients with H1N1 influenza and severe pneumonia, and >5000 individuals comprising patients with community-acquired mild lower respiratory tract infection and matched controls of Caucasian ancestry. We found evidence of an association between rs12252 rare allele homozygotes and susceptibility to mild influenza (in patients attending primary care) but could not confirm a previously reported association between this single-nucleotide polymorphism and susceptibility to severe H1N1 infection.

Keywords. genetics; H1N1; Influenza; LRTI; infectious disease; IFITM3; association study; Virus.

A recent functional genomic screen showed that host interferon-inducible transmembrane proteins 1, 2, and 3 (IFITM1, IFITM2, and IFITM3) block early stages of viral replication and thereby mediate cellular resistance to viruses, including influenza, West Nile, and dengue [1]. The mechanism by which IFITM proteins inhibit viral infection is not fully understood, but it has been shown, for example, that these proteins prevent the transfer of viral components to the cytosol by blocking membrane fusion [2]. Influenza A virus replication is persistent and associated with increased cell death and exaggerated proinflammatory responses in the lungs of mice lacking *Ifitm3* [3].

The 1000 Genomes project currently lists 15 nonsynonymous, 15 synonymous, 1 stop-site gaining, and 1 possible splice-site altering single-nucleotide polymorphism (SNP) in the *IFITM3* gene. The possible splice-site altering SNP (rs12252) is found close to a splice site for 1 transcript of the *IFITM3* gene [4]. Human genetic studies of the *IFITM3* gene have been limited, and no associations have been found with SNPs in the *IFITM3* gene in any published genome-wide association studies, according to the GWAS catalog [5].

Recently Everitt et al [3] reported a putative association between the C allele in *IFITM3* SNP rs12252 and susceptibility to severe H1N1 or seasonal influenza virus infection in patients from the United Kingdom. However, their study included only 53 patients with influenza, and the reported association relied on imputed control genotypes. Small sample sizes are prone to imprecisely estimate genotype frequencies, and imputed genotypes may be more inaccurate for rare SNPs such as *IFITM3* rs12252 [6] (which has a minor allele frequency of 3% in Europe [4]).

Another study analyzed the association between the rs12252 SNP and H1N1 influenza in China, where the frequency of this SNP is much higher [7]. This study found an association between C homozygotes and severe H1N1 infection when compared with either population controls from the 1000 Genomes Project or patients with mild H1N1 infection. This suggests that rs12252 associates with only severe H1N1 influenza infection. However, despite increased power due to the greater frequency of this SNP in China, interpretation of the results of this study is difficult since sample sizes were small (32 patients with severe and 51 with mild influenza).

We studied 2 separate cohorts to examine further this possible association with viral disease. Patients with severe H1N1 influenza requiring intensive care unit (ICU) admission for pneumonia (n = 34), patients with lower respiratory tract infection (LRTI) (n = 2730), and healthy controls matched to the patients with LRTI (n = 2623) were genotyped. The patients with severe H1N1 influenza were admitted to the ICU with community-acquired pneumonia and recruited to the UK-based Genomic Advances in Sepsis (GAinS) study [8]. The patients with LRTI and healthy controls were recruited from across Europe within the Genomics to combat Resistance

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against Antibiotics in Community acquired LRTI in Europe (GRACE) study [9].

METHODS

Genotyping Methods

Genotyping of rs12252 was performed by restriction fragment length polymorphism using primers CAGGAAAAGGAAA CTGTTGAGAACC(F) and CTCCTGGAGCCTCCTCCTCA (R) in standard polymerase chain reaction (PCR) conditions. Primers had 3′ penultimate base mismatches to *IFITM3* to ensure specific amplification of *IFITM3* rather than *IFITM2. MSc*I (New England Biolabs) was used to cut the PCR product in the presence of the wild-type T allele. Fragments with lengths of 572, 426, and/or 146 base pairs were visualized on agarose gels, according to the genotype of the sample. All minor homozygotes and heterozygotes were Sanger sequenced in the reverse direction to confirm their genotype, using the same primers.

Sample Collections

The GAinS study recruited adult patients admitted to ICUs in the United Kingdom with community-acquired pneumonia or fecal peritonitis [8]. Exclusion criteria for this study were inclusion in an interventional study of a novel intervention, immunosuppression (known regular systemic corticosteroid therapy, known regular therapy with other immunosuppressive agent, known presence of human immunodeficiency virus (HIV) positive or AIDS, neutrophil count $<1000\ mm^3$ owing to any cause; details reported elsewhere [8]), presence of a directive to withhold or withdraw life-sustaining treatment, or admission to ICU for palliative care only. Individuals with community-acquired pneumonia, H1N1 infection and self-reported Caucasian ancestry were included in the analysis presented here. All of these patients had chest radiographic evidence of pneumonia. None had malignant disease or liver cirrhosis, was receiving systemic steroids, or had significant premorbid exercise restriction. In contrast to the cohort studied by Everitt et al [3], none of the patients in the GAinS study was pregnant. Only 2 of them had chronic obstructive pulmonary disease, and 5 had asthma. Superadded or concurrent infections were identified in 7 patients (3 with *Staphylococcus aureus* and 1 each with *Staphylococcus hominis*, *Haemophilus influenzae*, *Mycoplasma pneumoniae,* and respiratory syncytial virus). All patients were mechanically ventilated except for 1.

GRACE [9] is a Europe-wide study of LRTI in primary care. Patients and controls used in this study were collected from 14 primary healthcare networks in 11 European countries, with patients matched to local controls by age, sex, and time of sampling. Subjects of non-European origin were excluded from the analysis. Eligible patients were aged ≥18 years, consulting for the first time with an acute cough (duration, ≤28 days) as the main symptom or acute LRTI as the primary diagnosis. None of the CC genotype patients with LRTI in this study were hospitalized. In house real-time PCR was performed to diagnose viral infection in 3 collaborating laboratories based on previous validation of the different nucleic acid amplification methods used in the respective laboratories [10].

Statistical Methods

All analyses included 2623 Caucasian European controls from GRACE. Two-tailed Fisher's exact test was used for the H1N1 analyses (SPSS software; version 18). GRACE patients and controls from across Europe were analyzed using logistic regression in PLINK software [11, 12], with country as a covariate. All Hardy-Weinberg equilibrium *P* values were calculated using PLINK software [11, 12].

RESULTS

Association analysis results between our cohort of patients with severe H1N1 and a large collection of controls, the largest available collection of directly genotyped Caucasians for rs12252 that we know of, showed no significant association (Table 1). We observed no CC homozygotes among our patients with severe H1N1 infection and nearly identical frequencies of TC heterozygotes in our patients and controls. However, association analysis between these controls and the combined patients with severe influenza (data from our study and Everitt et al [3]) suggests a recessive model of association (Table 1), rather than the additive model originally described [3]. Rare homozygotes are driving this association and show an odds ratio of 23.38, with no association for heterozygotes compared with major homozygotes (odds ratio, 1.05; 95% confidence interval, .48–2.30).

We extended our study to assess the possible role of *IFITM3* rs12252 in susceptibility to mild viral LRTI diagnosed in primary care (n = 1248), including patients with rhinovirus (n = 498), influenza (n = 240), coronavirus (n = 169), respiratory syncytial virus (n = 116), human metapneumovirus (n = 110), parainfluenza virus (n = 61), or coinfection with 2 of these viruses (n = 54). Comparing the *IFITM3* rs12252 genotypes of the combined virally infected primary care patients with those of the controls demonstrated an association for the recessive test ($P = .049$; Table 1) that was more significant in the subgroup of patients with disease due to influenza virus ($P = .025$; Table 1). As with severe H1N1, the best fitting model of association was recessive. When patients with noninfluenza viral infection were studied, no association was found (Table 1). No association was seen in rhinovirus or coronavirus specific analyses either (data not shown). We found no evidence of association between rs12252 and bacterial LRTI (n = 379; data not shown).

Table 1. Association Results for *IFITM3* SNP rs12252[a]

Association	GRACE Controls (n = 2623)	Patients by Study and Infection					
		Everitt et al [1] Influenza (n = 53)	GAinS H1N1 (n = 34)	GAinS H1N1 and Everitt et al [3] Influenza (n = 87)	GRACE Mild Viral Infection (n = 1248)	GRACE Mild Influenza (n = 259)	GRACE Noninfluenza Viral Infection (n = 989)
HWE *P* value	>.99	.000048	>.99	.0047	.007	.12	.0248
Genotype, No. (%)							
TT	2417 (92.1)	46 (86.8)	31 (91.2)	77 (88.5)	1160 (92.9)	235 (90.7)	925 (93.5)
CT	202 (7.7)	4 (7.4)	3 (8.8)	7 (8.0)	82 (6.6)	22 (8.5)	60 (6.1)
CC	4 (0.15)	3 (5.6)	0	3 (3.4)	6 (0.48)	2 (0.77)	4 (0.4)
Allelic *P* value	. . .	0.011[b]	0.753	0.032[b]	0.764	0.154	0.264
Allelic OR	. . .	2.498 (1.284–4.861)	1.107 (0.345–3.551)	1.936 (1.082–3.464)	0.960 (0.750–1.229)	1.358 (0.892–2.07)	0.8529 (0.6452–1.127)
Recessive model *P* value	. . .	2.4×10^{-4}[b]	>.99	0.001[b]	0.049[b]	0.025[b]	0.150
Recessive model OR	. . .	39.29 (8.567–180.137)	NA	23.38 (5.152–106.132)	3.59 (1.008–12.79)	7.126 (1.283–39.58)	2.778 (0.6901–11.19)

Abbreviations: GAinS, Genomic Advances in Sepsis; GRACE, Genomics to combat Resistance against Antibiotics in Community acquired LRTI in Europe; HWE, Hardy-Weinberg equilibrium; IFITM3, interferon-inducible transmembrane protein 3; NA, not applicable; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a] All analyses included 2623 Caucasian European controls from GRACE. Two-tailed Fisher's exact test was used for the H1N1 analyses (SPSS software, version 18). GRACE patients and controls from across Europe were analyzed using logistic regression in PLINK software [11, 12], with country as a covariate. The HWE *P* values were calculated using PLINK software [11, 12].

[b] $P < .05$

DISCUSSION

Without directly genotyped controls and large sample sizes, it is not possible to fully define genetic associations. We did not find an association between rs12252 and our patients with H1N1 influenza, probably owing to small sample size. However, by extending the case series of influenza patients reported by Everitt et al [3] and using our large collection of directly genotyped controls, we find a distinct genetic model for severe influenza susceptibility in humans. However, even with the combination of our genotyped patients with H1N1 influenza and the patients with influenza studied by Everitt et al [3] (85% of whom had H1N1 influenza), the number of patients in this study is small and the association relies on only 3 homozygotes genotyped by Everitt et al [3]. Not all of these 3 individuals have undergone population stratification analysis, and given the large differences in the allele frequency of this SNP between populations, population outliers could easily have biased this result. More patients with H1N1 influenza would be needed to better define this association in Europe.

Moreover, by analyzing large numbers of patients with community-acquired LRTIs and matched controls, we show the same recessive association for mild viral infections. We find the most significant association with susceptibility to mild influenza infection, in contrast to Zhang et al [7], who found an association with severe but not mild influenza. The interferon-pathway gene *IFITM3* therefore seems to act as a susceptibility locus for influenza infection in humans, encompassing both very mild and life-threatening disease. We found no evidence that this SNP is associated with mild bacterial infection, which is unsurprising because IFITM3 has not been implicated in the control of bacterial infection. Although IFITM3 has been shown to mediate infection by multiple viruses, including influenza A, HIV-1, yellow fever virus, and West Nile virus [1, 2, 13–15], of the pathogens we have studied, the association seemed to be specific for influenza susceptibility. Therefore future studies with other large viral sample sets are necessary to further define this genetic association.

One limitation of our study is that population stratification analysis has not been performed for our samples. Whole-genome genotyping has not been performed to make this possible. Self-reported ancestry has been used to remove non-Caucasian subjects from the analysis.

There have been many case-control studies of severe viral and bacterial infectious disease, which are almost always recruited in a hospital setting. However, there have been very few studies of the genetics of susceptibility to common mild infections that are generally managed in primary care. This is the first large genetic study, of which we are aware, assessing susceptibility to mild LRTIs in a primary care (general practice) setting. Our data suggest that larger studies of mild infection phenotypes in addition to severe cases could help distinguish

between genetic determinants of initial infection as opposed to severe consequences of infection. Moreover, the large variety of microbial causes of common infections in primary care may allow more efficient identification of genetic loci that affect the risk of infection by multiple pathogens.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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