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Genetic variants associated with severe pneumonia in A/H1N1 influenza infection

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ABSTRACT: The A/H1N1 influenza strain isolated in Mexico in 2009 caused severe pulmonary illness in a small number of exposed individuals. Our objective was to determine the influence of genetic factors on their susceptibility.

We carried out a case–control association study genotyping 91 patients with confirmed severe pneumonia from A/H1N1 infection and 98 exposed but asymptomatic household contacts, using the HumanCVD BeadChip (Illumina, San Diego, CA, USA).

Four risk single-nucleotide polymorphisms were significantly ($p<0.0001$) associated with severe pneumonia: rs1801274 (Fc fragment of immunoglobulin G, low-affinity IIA, receptor (*FCGR2A*) gene, chromosome 1; OR 2.68, 95% CI 1.69–4.25); rs9856661 (gene unknown, chromosome 3; OR 2.62, 95% CI 1.64–4.18); rs8070740 (RPA interacting protein (*RPAIN*) gene, chromosome 17; OR 2.67, 95% CI 1.63–4.39); and rs3786054 (complement component 1, q subcomponent binding protein (*C1QBP*) gene, chromosome 17; OR 3.13, 95% CI 1.89–5.17). All SNP associations remained significant after adjustment for sex and comorbidities. The SNPs on chromosome 17 were in linkage disequilibrium.

These findings revealed that gene polymorphisms located in chromosomes 1 and 17 might influence susceptibility to development of severe pneumonia in A/H1N1 infection. Two of these SNPs are mapped within genes (*FCGR2A*, *C1QBP*) involved in the handling of immune complexes and complement activation, respectively, suggesting that these genes may confer risk due to increased activation of host immunity.

KEYWORDS: A/H1N1, genetic susceptibility, influenza, Mexicans, single-nucleotide polymorphisms, viral pneumonia

The A/H1N1 influenza virus has a mixture of genes from Eurasian swine, human and avian influenza viruses [1]. The A/H1N1 strain isolated in Mexico City in 2009 caused severe pulmonary illness in people from many countries. The clinical and demographic characteristics of the cases with severe pneumonia at the beginning of the outbreak in Mexico have been reported [2]. However, the mechanisms responsible for the development of severe pneumonia associated with A/H1N1 infection have not been well defined. In contrast to seasonal influenza, the serious illnesses caused by pandemic A/H1N1 occurred primarily in young adults, and ~90% of deaths occurred in those aged <65 yrs [3]. Although host immune responses play crucial roles in defence against influenza, they have been implicated in the pathology of certain influenza strains, such as the avian influenza A H5N1 and the 1918 H1N1 influenza [4]. One possible explanation for the predilection of severe A/H1N1 infection for children and nonelderly adults is that increased activation of the immune system contributes to the pathogenesis and poor clinical outcomes of the severe form of A/H1N1 disease. In support of this hypothesis, immune complex deposition and complement activation in the respiratory tract have recently been implicated in the ability of pandemic 2009 influenza A/H1N1 virus to cause severe disease in middle-aged adults without pre-existing comorbidities [5].

Host genetic factors may affect the development and progression of many infectious diseases [6]. Genetic polymorphisms appear to be important in explaining variations in immune response to

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influenza viruses, and specific genes may affect disease susceptibility or severity [7]. In this article, we describe a case–control association study to identify genetic polymorphisms associated with increased risk of severe A/H1N1 pneumonia, using HumanCVD BeadChips (Illumina, San Diego, CA, USA) containing more than 48,000 single-nucleotide polymorphism (SNP) probes targeting ~2,100 candidate genes [8]. To our knowledge, this is the first study of genetic determinants of risk for severe disease associated with the pandemic A/H1N1 virus. We studied 189 Mexican individuals, comprising 91 with confirmed severe pneumonia from A/H1N1 infection and 98 household contacts exposed to the A/H1N1 virus who did not develop pneumonia.

METHODS

Study population

91 patients with A/H1N1 who developed severe pneumonia (56 male and 35 female), and 98 household contacts (35 male and 63 female), were included in the study. Patients with A/H1N1 infection who developed severe pneumonia were hospitalised in the influenza containment area of the emergency room and in the intensive care unit of the National Institute of Respiratory Diseases (INER) during the first outbreak in Mexico City between May and October 2009. The clinical criteria for the recruitment of patients with severe pneumonia were: 1) confirmed acute A/H1N1 infection by RT-PCR; 2) confirmed pneumonia with bilateral opacities predominantly in basal areas on high-resolution computed tomography (fig. 1); and 3) Kirby index (arterial oxygen tension (P_{a,O_2})/inspiratory oxygen fraction (F_{I,O_2})) <250. 42% of these patients had a Kirby index <200 and required mechanical ventilation. Pregnant females were not included in this study.

As a control group, we recruited 98 asymptomatic household contacts of the confirmed cases with mean ± SD age 38.2 ± 15.0 yrs. Only unrelated contacts were included in this study (*e.g.* spouse, home workers or friends). They were in close contact with patients when the latter exhibited acute respiratory illness. None of these household contacts developed respiratory illness. Importantly, 76.5% of the household contacts exhibited significant titres of specific anti-A/H1N1 antibodies (>1:16), supporting the fact that they were in contact with the A/H1N1 virus.

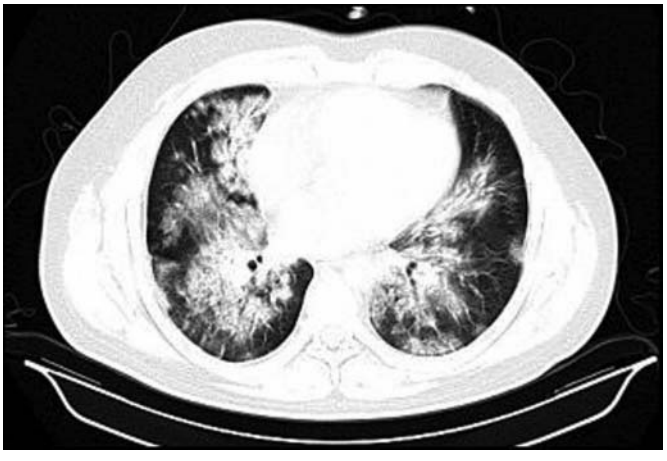

FIGURE 1. High-resolution computed tomography scan of a patient with severe pneumonia associated with influenza A/H1N1 infection, showing multifocal ground-glass attenuation and consolidations and reticular opacities.

The Institutional Review Board of the INER (Mexico City, Mexico) reviewed and approved the protocols for genetic studies under which all subjects were recruited. All subjects provided written informed consent for genetic studies, and they authorised the storage of their genomic DNA at the INER repositories for this and future studies. After obtaining signed informed consent letters from patients and household contacts, we performed venipuncture to obtain 10 mL peripheral blood.

For this study, we enrolled only individuals whose last two generations were born in Mexico (Mexican Mestizos). We have studied several genetic polymorphic markers in Mexican Mestizos, and the admixture estimations have revealed an important contribution of Amerindian (~60%) and Caucasian (30%) genes, with only 5–10% African genes [9].

A/H1N1 virus detection

Nasal swab samples were obtained from hospitalised patients at the INER following the criteria described by the US Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO). RNA isolation was performed using the viral RNA mini kit (Qiagen Westburg, Leusden, the Netherlands). Detection of A/H1N1 influenza viruses in respiratory specimens was assessed by real-time RT-PCR according to CDC and WHO guidelines.

Anti-A/H1N1 antibody titres

The titres of serum anti-A/H1N1 antibodies were measured using a previously described haemagglutination inhibition (HAI) technique [10]. Briefly, serially diluted aliquots of serum samples (25 μL) in PBS were mixed with 25-μL aliquots of the A/H1N1 virus strain isolated at INER (corresponding to four haemagglutination units). The serum/virus dilutions were incubated for 30 min at room temperature. 50 μL of 0.5% chicken erythrocytes were added and after 30 min the HAI activity was evaluated. The serum HAI antibody titre was established as the reciprocal of the last serum dilution with no haemagglutination activity. Those individuals with titres greater than 1:16 were considered positive for A/H1N1 infection/exposure.

DNA isolation and SNP genotyping

Genomic DNA was isolated from EDTA-anticoagulated peripheral blood using Qiagen blood mini kits (Qiagen, Chatsworth, CA, USA), and was stored at -80°C. We used the ITMAT-Broad-CARe or ''IBC array'' (HumanCVD BeadChip; Illumina [7]), which incorporates ~50,000 SNPs, to efficiently capture genetic diversity across >2,000 genic regions related to cardiovascular, inflammatory and metabolic phenotypes. Genetic variation within the majority of these regions is captured at density equal to or greater than that afforded by genome-wide genotyping products [8].

Quality-control measures were conducted using the software package PLINK v1.07 [11]. For SNP quality control, we removed 1,014 SNPs on sex chromosomes, 18,895 SNPs with minor allele frequency <0.05, 1,038 SNPs with missing proportion >10%, and 120 SNPs with Hardy–Weinberg test $p \leqslant 0.001$ in controls, leaving 28,368 SNPs for analysis. All individuals in the data set have genotype missing rates <10%. The genotyping of the

functional polymorphism rs1801274 at the *FCGR2A* gene (A/G substitution) was confirmed using a validated TaqMan 5′ nuclease assay (Assay ID: C___9077561_20; Applied Biosystems, Foster City, CA, USA). The final reaction volume was 25 μL, containing 15 ng genomic DNA, 12.5 μL 2× TaqMan Universal PCR Master Mix, 0.625 μL 40× Assay Mix and 8.8 μL DNA/RNAse-free water. PCR conditions were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min, then finally 60°C for 30 s. All PCR reactions were performed using 96-well optical plates in a Step-One Plus real-time PCR system (Applied Biosystems).

Statistical analyses

Demographic and clinical characteristics were tested with the unpaired t-test or Fisher's exact method using the SAS software v9.1.3 (SAS Institute, Cary, NC, USA). All genetic association analyses were performed using PLINK v1.07 [11]. Both univariate and multivariate unconditional logistic models were fitted to test the association between severe pneumonia and each SNP assuming an additive genetic effect, in which an SNP is coded as 0, 1 or 2 by the number of minor alleles the individual carries. Odds ratios (ORs) were calculated by the exponential of the estimated coefficient of the SNP in the logistic model, as well as their 95% confidence intervals. We used two-sided tests in this study.

The HumanCVD chip contains ~50,000 SNPs. A standard Bonferroni correction would yield a significance level of $\sim 1 \times 10^{-6}$, resulting in very conservative results of significance tests. However, since the HumanCVD array has a dense gene-centric design, some studies have used a less stringent level of 1×10^{-5} [12]. In this study, we used a significance level of 1×10^{-4}, because our sample size was limited, and we sought to identify more potential susceptibility SNPs. Power calculation showed that this significance level would yield a power of 77% to detect an effect size of OR 3.0 given the minor allele frequency of 20%, and a power of 48% to detect an effect size of OR 2.5. To adjust for type I error, we also used the false discovery rate (FDR) to evaluate the proportion of false positives among our findings.

Population stratification was assessed using the 1,443 ancestry information marker SNPs that the CVD chip contains. We performed a principal component (PC) analysis using the software package EIGENSTRAT [13] and extracted the first six PCs based on Tracy–Widom statistics. The six PCs were then used as covariates to adjust for population stratification.

RESULTS

Clinical features

Demographic and clinical characteristics of patients are summarised in table 1. Patients with severe A/H1N1 pneumonia were predominantly male (61.5%, $p<0.0005$ compared with household controls) (table 1). The mean time of evolution of respiratory disease in A/H1N1 patients was 9 days. The most common symptoms were fever (89%), dry cough (87%) and dyspnoea (80%). Leukocytosis was detected in 61% of the patients. Lymphopenia was present in 38% of the patients and high lactate dehydrogenase levels (>1,000 $U \cdot L^{-1}$) were found in 29%. Bilateral radiographic opacities and hypoxaemia were observed in all patients. No significant differences were observed in age between cases and controls, or in the prevalence of comorbidities, including obesity, diabetes, arterial hypertension, chronic obstructive pulmonary disease, asthma, liver cirrhosis and intestinal, renal, heart, brain and vascular diseases. The frequency of cigarette smoking was higher in patients ($p<0.0001$), while the prevalence of influenza vaccination was higher in the group of contacts without pneumonia ($p<0.0001$). 42 patients with severe pneumonia (46.2%) exhibited a $P_{a,O_2}/F_{I,O_2}$ index <200 and required mechanical ventilation (table 1).

TABLE 1 Demographic and clinical characteristics of patients with pneumonia associated with A/H1N1 virus infection

Variable	Severe pneumonia patients	Household contacts	p-value
Subjects n	91	98	
Age yrs	38.3±15.6	38.2±15.0	0.9618
Sex male	56 (61.54)	35 (35.71)	0.0005
Obesity	45 (51.72)	42 (47.73)	0.6512
Diabetes mellitus	9 (10.00)	4 (4.49)	0.2488
Arterial hypertension	14 (15.38)	17 (19.10)	0.5574
Tobacco use	38 (41.76)	20 (22.73)	0.0070
Seasonal influenza vaccination	1 (1.10)	23 (23.71)	<0.0001
Healthcare worker	1 (1.10)	0	0.4815
Mechanical ventilation	42 (46.15)	0	<0.0001
Kirby index ($P_{a,O_2}/F_{I,O_2}$)	207.6±77.1		
COPD	1 (1.10)	0	1.0000
Asthma	5 (5.56)	1 (1.12)	0.2108
Intestinal disease	5 (5.49)	1 (1.12)	0.2110
Cerebrovascular disease	3 (3.30)	0	0.2459
Immunosuppression	3 (3.30)	2 (2.25)	1.0000

Data are presented as mean±SD or n (%), unless otherwise stated. P_{a,O_2}: arterial oxygen tension; F_{I,O_2}: inspiratory oxygen fraction; COPD: chronic obstructive pulmonary disease.

Four SNPs were associated with susceptibility to severe pneumonia

The results of single-SNP analysis are shown in table 2, and the corresponding distribution of $-\log_{10}(p)$ of all SNPs (Manhattan Plot) is illustrated in online supplementary figure 1S. Four risk SNPs in genes located on three chromosomes were identified with significant p-values <0.0001. The SNPs associated with the development of severe pneumonia were rs1801274 (Fc fragment of immunoglobulin (Ig)G, low-affinity IIA, receptor (*FCGR2A*) gene, chromosome 1; OR 2.68, 95% CI 1.69–4.25); rs9856661 (gene unknown, chromosome 3; OR 2.62, 95% CI 1.64–4.18); rs8070740 (RPA interacting protein (*RPAIN*) gene, chromosome 17; OR 2.67, 95% CI 1.63–4.39); and rs3786054 (complement component 1, q subcomponent binding protein (*C1QBP*) gene, chromosome 17; OR 3.13, 95% CI 1.89–5.17).

The SNP rs1801274 codes for a nonsynonymous change in the amino acid sequence encoded by the *FCGR2A* gene at position 131 (His131Arg). The genotype frequency of the homozygous His131 genotype was significantly increased in patients with severe pneumonia (36.6%) when compared with household contacts who did not develop respiratory illness (13.2%) ($p=0.0003$, OR 3.79, 95% CI 1.74–8.34). In contrast, the frequency

TABLE 2 Results of individual single nucleotide polymorphism (SNP) analysis

SNP	Minor allele	Chromosome	Location bp	Genotype frequencies#		OR (95% CI)	p-value	FDR	Gene name
				Severe pneumonia	Contacts				
rs1801274	A	1	159746369	33/46/12	13/51/34	2.68 (1.69–4.25)	2.56×10^{-5}	0.36	*FCGR2A*
rs9856661	C	3	54052296	26/48/17	8/51/39	2.62 (1.64–4.18)	5.41×10^{-5}	0.51	Unknown
rs8070740	G	17	5272620	17/61/13	10/45/43	2.67 (1.63–4.39)	9.49×10^{-5}	0.56	*RPAIN*
rs3786054	A	17	5279783	16/54/21	5/39/54	3.13 (1.89–5.17)	7.90×10^{-6}	0.22	*C1QBP*

bp: base pair; FDR: false discovery rate. #: genotype frequencies in patients with severe pneumonia (n=91) and household contacts (n=98) are represented as: minor allele homozygotes/major allele and minor allele heterozygotes/major allele homozygotes.

of the homozygous genotype Arg131 was higher in the household contacts ($p<0.05$). The genotype of this functional change in the *FCGR2A* gene was corroborated by real-time PCR.

The four SNPs associated with severe disease remained significant after adjusting for population stratification (data not shown). Another SNP, rs3744714 (*DHX33*) on chromosome 17, was revealed as significant after adjusting for sex, influenza vaccination, hypertension, obesity and diabetes. Interestingly, three of the genes with risk SNPs found in this study, *RPAIN*, *C1QBP* and *DHX33*, are located in close proximity to each other on the short arm of chromosome 17 (17p13.2–13.3). These three significant SNPs, rs8070740, rs3786054 and rs3744714 on chromosome 17, were all in high linkage disequilibrium (fig. 2 and online supplementary fig. 2S).

After adjusting for obesity, diabetes and arterial hypertension, all five risk SNPs identified remained significantly associated with susceptibility to severe pneumonia ($p<0.0001$) (table 3). After adjusting for age, sex and smoking, the SNPs in *FCGR2A* (rs1801274, chromosome 1) and *C1QBP* (rs3786054, chromosome 17) remained significantly associated with severe pneumonia at the 5×10^{-5} level.

We also matched cases and controls using the criteria of the same sex and age ± 5 yrs; 68 matched pairs were found. Conditional logistic analysis based on these 68 pairs showed similar estimated ORs to those in our unconditional analysis (online supplementary table 1S).

DISCUSSION

Host genetic factors are likely to influence resistance or susceptibility to pandemic A/H1N1 virus infection, as well as to the development of severe pneumonia. This exploratory study provides evidence that genetic factors played an important role in determining the susceptibility of Mexican Mestizo individuals to the development of severe pneumonia in the first outbreak of A/H1N1 infection in Mexico City between May and October 2009. We found significant associations of five SNPs (rs1801274, rs9856661, rs8070740, rs3786054, and rs3744714) located on chromosomes 1, 3 and 17 with the development of severe pneumonia in patients with A/H1N1 virus infection.

Three of these SNPs occur in genes (*FCGR2A*, *C1QBP* and *RPAIN*) that may affect either host immune responses to, or replication of, the A/H1N1 influenza virus. Immune complexes and complement activation have recently been implicated in the pathogenesis of severe disease in A/H1N1-infected middle-aged adults [5]. Severe disease in this pandemic was found to be associated with high titres of low-avidity, non-protective anti-influenza antibodies, leading to immune complex deposition and complement activation in the respiratory tract [5]. Notably, one of the genes in which we found a risk SNP, *FCGR2A*, affects handling of immune complexes, and another, *C1QBP*, can activate complement.

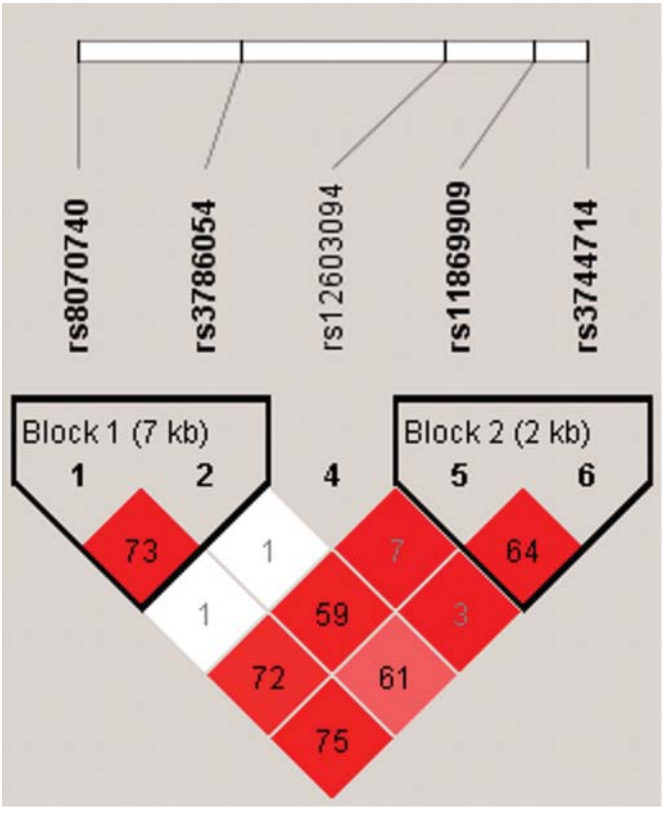

FIGURE 2. Linkage disequilibrium (LD) structure of the chromosome 17 region containing the significant single-nucleotide polymorphisms associated with severe pneumonia. The LD plot was generated using Haploview v4.2 (Broad Institute, Cambridge, MA, USA). The degree of pairwise LD (r^2) is also shown in each block.

TABLE 3 Risk single-nucleotide polymorphisms (SNPs) associated with susceptibility to severe pneumonia ($p<0.0001$) after multivariable analysis[#]

Chromosome	SNP	Location bp	n	OR (95% CI)	p-value
1	rs1801274	159746369	173	3.21(1.93–5.33)	6.45×10^{-6}
3	rs9856661	54052296	174	2.59(1.57–4.27)	2.04×10^{-4}
17	rs8070740	5272620	174	2.94(1.72–5.02)	7.94×10^{-5}
17	rs3786054	5279783	174	3.82(2.19–6.67)	2.39×10^{-6}
17	rs3744714	5294801	174	2.92(1.73–4.92)	6.20×10^{-5}

bp: base pair. [#]: Covariates used in the analysis were obesity, diabetes, arterial hypertension, age, sex and smoking.

The *FCGR2A* gene encodes the Fcγ receptor IIA (FcγRIIA), which binds immune complexes with high avidity [14]. SNP rs1801274 (A/G) in the *FCGR2A* gene results in a nonsynonymous change in the amino acid sequence of FcγRIIA at position 131 (His131Arg). The homozygous His131 genotype (A/A) was significantly enriched in our patients with severe pneumonia compared with household contacts who did not develop respiratory illness despite A/H1N1 exposure. This single amino acid change at position 131 is known to have important functional consequences for FcγRIIA [15]. The His131 allele of *FCGR2A* (*FcγRIIA-H131*) has greater affinity than the Arg131 allele (*FcγRIIA-R131*) for all human IgG subclasses [15, 16]. The affinity of FcγRIIA-R131 for IgG_2 is particularly reduced, and FcγRIIa-H131 is the only human Fcγ receptor that recognises this IgG subclass efficiently [15, 16]. Immunoglobulin engagement of activating-type Fc receptors such as FcγRIIA induces multiple pro-inflammatory events, including immune cell degranulation and transcriptional activation of cytokine-encoding genes [16]. FcγRIIA alleles have been demonstrated to modulate the ability of phagocytes to bind and internalise IgG-opsonised particles, with FcγRIIA-H131 conferring greater phagocytic function [15, 16].

The effect of FcγRIIA alleles on immune complex-driven pathology may be complex and bidirectional. To the extent that the increased phagocytic function conferred by FcγRIIA-H131 leads to increased clearance of these complexes, this allele could be expected to protect against immune complex diseases. However, to the extent that the increased IgG affinity of FcγRIIA-H131 leads to increased inflammatory cascade activation in response to immune complexes, this allele could be expected to promote immune complex-driven pathologies. There is evidence for both protective and harmful effects of FcγRIIA alleles in other diseases. The FcγRIIA-H131 allele has been found to be under-represented in systemic lupus erythematosus, consistent with this allele having on balance a protective effect in this prototypic human immune complex disease [17]. In contrast, the FcγRIIA-H131 allele has been found to be over-represented in dengue virus infections with severe clinical courses, either dengue fever or dengue haemorrhagic fever, compared to subclinical infections [18, 19]. Based on our finding that the homozygous *FcγRIIA-H131* genotype was significantly enriched in A/H1N1 patients with severe pneumonia, we hypothesise that this allele also has an overall harmful effect in A/H1N1 infection, possibly due to increased inflammatory cascade activation in response to immune complex deposition in the respiratory tract.

On chromosome 17, three SNPs were significantly associated with severe disease. The strongest association after multivariable analysis (table 3) was observed with the SNP rs3786054 ($p=2.39\times10^{-6}$, OR 3.82), located in the *C1QBP* gene, which encodes the protein gC1qR. gC1qR was originally identified as a high-affinity receptor for C1q [20]. C1q is the first subcomponent of the C1 complex of the classical pathway of complement activation [21], and gC1qR can activate this pathway [20, 22]. gC1qR may also contribute to the activation of the classical pathway of complement by the surface of activated platelets [23]. We hypothesise that the risk allele of *C1QBP* associated with severe A/H1N1 disease is associated with increased complement activation. In addition to C1q, gC1qR is also able to bind several other biologically important plasma ligands, including high-molecular-weight kininogen (HK) and factor XII (FXII), two of the four proteins of the kallikrein/kinin system of contact activation [24]. Incubation of FXII, prekallikrein, and HK with gC1qR converts prekallikrein to kallikrein, which in turn is required for kinin generation [24]. In addition to its ability to activate complement, cC1qR can therefore also amplify inflammation by facilitating the assembly of contact activation proteins leading to generation of bradykinin.

Another polymorphism associated with severe pneumonia due to A/H1N1 infection, rs8070740, is located in the 3′-untranslated region of the gene *RPAIN*, which is also known as *hRIP* (human Rev-interacting protein). This gene has been mapped to human chromosome 17p13, and encodes a nucleoporin that is involved in RNA trafficking and localisation [25]. hRIP acts as a cellular co-factor required for the export of HIV RNAs from the nucleus of infected cells to the cytoplasm, a process mediated by the HIV-1 regulatory protein Rev that is essential for HIV-1 replication [26]. Export of influenza RNAs from the nucleus of infected cells to the cytoplasm is mediated by the influenza-encoded nuclear export protein (NEP), previously named NS2. hRIP also interacts strongly with influenza NEP, and in so doing, hRIP may similarly be a required cellular co-factor for influenza replication [27]. We hypothesise that the risk allele of *hRIP/RPAIN* associated with severe A/H1N1 disease is associated with increased influenza replication. Interestingly, after adjusting for obesity, diabetes and hypertension, another SNP, rs3744714, was revealed to be significantly associated with the development of severe pneumonia in A/H1N1-infected persons. This SNP is located in the intronic region of the DEAH (Asp-Glu-Ala-His) box polypeptide 33 *(DHX33*) gene. The *DHX33* gene encodes a member of the DEAD box proteins, which are putative RNA helicases, but the

function of this particular protein is unknown. Notably, *C1QBP*, *RPAIN* and *DHX33* are located in close proximity to each other on the short arm of chromosome 17, and our results showed strong linkage disequilibrium between the disease-associated SNPs in these genes. Our observation of a stronger association of severe A/H1N1 disease with the SNP located within the *C1QBP* gene than with the SNPs in the *RPAIN* and *DHX33* genes suggests that the *C1QBP* SNP is driving the association between severe pneumonia and this region of chromosome 17, and that the other two SNPs may be acting as neighbouring markers of the real susceptibility gene polymorphism.

The ability of host genetic factors to influence susceptibility to, and clinical progression of, human infectious diseases has been investigated extensively. In the case of influenza A, wide variation in the susceptibility of different inbred laboratory strains of mice to infection indicates that the genetic background of the host makes major contributions to influenza A virus infections [28]. Nevertheless, little information is available on human genetic variation that may influence susceptibility to and severity of influenza virus infections [7]. A study of 100 candidate influenza susceptibility genes based on their potential role in the pathogenesis of influenza A infection has recently been suggested [29]. To our knowledge, our study is the first to investigate the influence of host genetic factors on the severity of the influenza infection in humans, and to do so by investigating a large number of genes in an unbiased fashion. To follow-up on our identification of risk genes for severe disease, functional studies will be needed to further investigate the role of these genes in the pathogenesis or clinical course of severe pneumonia associated with A/H1N1 infection.

In addition, our study is the first analysis of the IBC-CVD array in samples from a Mexican Mestizo population. Our results may, therefore, also contribute to future determinations of the frequencies of disease-associated genotypes in other inflammatory and metabolic disorders that are common in Mexicans and other admixed American ethnic groups.

Our study has some limitations, including its relatively small sample size, restricted by the study's focus on patients with severe pneumonia who presented during a short-duration outbreak. In addition, we chose not to include patients with severe pneumonia that was probably associated with A/H1N1 infection but without viral corroboration. With this stringently defined number of cases, in order to identify more potentially biologically important SNPs, we used a significance level of 1×10^{-4} to achieve an ~80% power. In this context, we cannot rule out the possibility that one or more of these SNPs may be a false positive, as the least FDR q value is 0.22 for rs3786054.

A second limitation is our inability to include a replication cohort, despite contacting investigators in other countries that had substantial numbers of A/H1N1 cases. In the context of the public health emergency that the A/H1N1 pandemic represented, neither the US ARDSNet nor the National Influenza A Pandemic (H1N1) 2009 Clinical Investigation Group of China [30] were able to archive the blood samples from patients with A/H1N1-associated severe pneumonia that we would have required to use for a replication cohort (personal communications, B.T. Thompson (Boston, MA, USA) and C. Wang (Beijing, China), respectively).

In summary, our study suggests that several polymorphisms might contribute to the risk of developing severe pneumonia in persons infected with the A/H1N1 influenza virus. Although our findings need to be replicated in other populations, three of the genes identified have functions that could plausibly influence susceptibility to and/or severity of A/H1N1 infection. Studies of the proteins encoded by *FCGR2A* and *C1QBP* suggest that these genes may be involved in the host immune response to A/H1N1 infection, whereas *RPAIN* might influence the ability of A/H1N1 to replicate in host cells. As noted, our identification of *FCGR2A*, a gene whose product affects handling of immune complexes, and *C1QBP*, whose product can activate complement, are particularly interesting in light of recent data implicating immune complexes and complement activation in severe A/H1N1 disease [5]. In the case of *FCGR2A*, we found that severe A/H1N1 disease is associated with the allele that is related to increased immune function, suggesting that this gene may confer risk for severe pneumonia due to increased activation of host immunity.

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STATEMENT OF INTEREST

None declared.

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The authors' contributions were as follows. J. Zúñiga, D. Christiani, Y. Zhao, G. Vargas, A. Tager, A. Pardo and M. Selman contributed to study conception and design, SNP data analyses and interpretation, acquisition of data, statistical analyses, obtaining funding and drafting the manuscript. J. Zuñiga, I. Buendía, J. Romo, D. Torres, L. Jiménez-Alvarez, G. Ramírez and A. Cruz recruited the A/H1N1 patients and performed the acquisition of clinical data, analysis and interpretation of clinical data, and contributed to the sampling of A/H1N1 patients and household control subjects, A/H1N1 infection RT-PCR diagnosis and comorbidity diagnosis, anti-A/H1N1 antibody titre measurement and DNA purification. C-C. Sheu and L. Su performed the genotyping, and Y. Zhao and F. Chen performed the statistical genetics analyses. M. Selman and D. Christiani had full access to all of the data in the study and take responsibility for the report.

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