

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

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**Abstract**

**Objective.** 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.

**Methods.** 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).

**Results.** Four risk SNPs were significantly ( $p < 0.0001$ ) associated with severe pneumonia: rs1801274 [Fc fragment of IgG, 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 (*CIQBP*) gene, chromosome 17, OR=3.13, 95%CI=1.89-5.17]. All SNP's remained significant after adjustment for gender and comorbidities. The SNPs on chromosome 17 were in linkage disequilibrium.

**Conclusions.** 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*, *CIQBP*) 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.

**Key words:** A/H1N1, Genetic susceptibility, Mexicans, Influenza, Single nucleotide polymorphisms, viral pneumonia.

## **Introduction**

The A/H1N1 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 different 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 approximately 90% of deaths occurred in those under 65 years of age [3]. Although host immune responses play crucial roles in defense 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 H1N1 influenza A to cause severe disease in middle-aged adults without preexisting 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 influenza viruses, and specific genes may affect disease susceptibility or severity [7]. In this work, we performed a case-control association study to identify genetic polymorphisms associated with increased risk of severe A/H1N1 pneumonia, using HumanCVD BeadChips (Illumina) containing more than 48,000 single nucleotide polymorphisms (SNPs) 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, including 91 with confirmed severe pneumonia from A/H1N1 infection and 98 household contacts exposed to the A/H1N1 virus that did not develop pneumonia.

## Methods

### Study population

Ninety-one 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 hospitalized in the influenza containment area of the emergency room and in the ICU 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 (**Figure 1**); and 3) Kirby index ( $\text{PaO}_2/\text{FiO}_2$ )  $<250$ . Forty two percent of these patients had a Kirby index  $<200$  and required mechanical ventilation. Pregnant women were not included in this study.

As a control group, we recruited 98 asymptomatic household contacts of the confirmed cases with mean age  $\pm$  SD of  $38.2 \pm 15.0$  years. 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 titers of specific anti-A/H1N1 antibodies ( $> 1:16$ ), supporting the fact that they were in contact with the A/H1N1 virus.

The Institutional Review Board of the National Institute of Respiratory Diseases 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 authorized the storage of their genomic DNA at The National Institute of Respiratory Diseases repositories for this and future studies. After obtaining the signed informed

consent letter from patients and household contacts, we performed venipuncture to obtain 10 ml of peripheral blood.

For this study we enrolled only those 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 genes (30%), and only 5-10% of African genes [9].

### **A/H1N1 virus detection**

Nasal swab samples were obtained from hospitalized patients at the INER following the criteria described by the 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 with CDC and WHO guidelines.

### **Anti-A/H1N1 antibody titers**

The titers of serum anti-A/H1N1 antibodies were measured using a previously described haemagglutination inhibition technique (HAI) [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 our Institute (corresponding to four haemagglutination units). The serum-virus dilutions were incubated for 30 min at room temperature. Fifty µl of 0.5% chicken erythrocytes were added and after 30 min the HAI activity was evaluated. The serum HAI antibody titer was established as the reciprocal of the last serum dilution

with no haemagglutination activity. Those individuals with titers 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), and was stored at -80°C. We used the ITMAT-Broad-CARe or ‘IBC array’ (CVD BeadChip [Illumina, San Diego, CA][7]), which incorporates approximately 50,000 SNPs to efficiently capture genetic diversity across over 2,000 genic regions related to cardiovascular, inflammatory and metabolic phenotypes. Genetic variation within the majority of these regions is captured at density equal or greater than that afforded by genome-wide genotyping products [8].

Quality control measures were conducted using the software package PLINK version 1.07 [11]. For SNP quality control, we removed 1014 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  $P \leq 0.001$  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). The final volume reaction was 25µl, containing 15 ng of genomic DNA, 12.5µl of 2X TaqMan Universal PCR Master Mix, with 0.625µl of 40X Assay Mix and 8.8µl of DNA/RNase free water. PCR conditions were as follows: Hold 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min and hold 60°C 30 s. All PCR reactions were performed using 96-well optical plates in a Step-One Plus real time PCR system (*Applied Biosystems*, Foster City, CA).

## Statistical analyses

Demographic and clinical characteristics were tested with Student's *t*-test or Fisher's exact method using the Statistical Analysis System (SAS) software v 9.1.3 (SAS Institute, Cary, NC). All genetic association analyses were performed using PLINK 1.07 [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 SNP is coded as 0, 1 or 2 by the number of minor alleles the individual carries. Odds ratios (OR) were calculated by the exponential of the estimated coefficient of SNP in the logistic model, as well as their 95% confidence intervals (CI). We used two sided tests in this study.

The Human CVD chip contains approximately 50,000 SNPs. A standard Bonferroni correction would yield a significance level of approximately 1E-6, resulting in very conservative results of significance tests. However, since the Human CVD array has a dense gene-centric design, some studies used a less stringent level of 1E-5 [12]. In this study, we used a significance level of 1E-4 because our sample size is 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 MAF 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 (AIM) SNPs that the CVD chip contains. We performed a principal component analysis (PCA) using the software EIGENSTRAT and extracted the first 6 principal components (PCs) based on Tracy-Widom statistics [13]. The 6 PCs were then used as covariates to adjust for population stratification.



## Results

### Clinical features

Demographic and clinical characteristics of patients are summarized 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 dyspnea (80%). Leukocytosis was detected in 61% of the patients. Lymphopenia was present in 38% of the patients and high LDH levels ( $>1000\text{U/L}$ ) were found in 29%. Bilateral radiographic opacities and hypoxemia were observed in all the patients. No significant differences were observed in age between cases and controls, or in the prevalence of co-morbidities, including obesity, diabetes, arterial hypertension, COPD, asthma, liver cirrhosis, intestinal, renal, heart, brain and vascular diseases. The frequency of cigarette smokers was higher in patients ( $p < 0.0001$ ), while the prevalence of flu vaccination was higher in the group of contacts without pneumonia ( $p < 0.0001$ ). Forty-two patients with severe pneumonia (46.2%) exhibited a  $\text{PaO}_2/\text{FiO}_2$  index below 200 and required mechanical ventilation (**Table 1**).

### Four SNPs were associated with susceptibility to severe pneumonia

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 **Figure 1S** (see online supplementary material). Four risk SNPs in genes located on 3 chromosomes were identified with significant  $p$  values  $< 0.0001$ . The SNPs associated with the development of severe pneumonia were rs1801274 [Fc fragment of IgG, 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 (*CIQBP*) gene, chromosome 17, OR= 3.13, 95% CI= 1.89-5.17].

The SNP rs1801274 codes for a non-synonymous change in the amino acid sequence encoded by the *FCGR2A* gene at position 131 (His131Arg). The genotype frequency of the homozygous His131 allele 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 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 (not shown). Another SNP, rs3744714 (*DHX33*) on chromosome 17 was revealed as significant after adjusting for gender, influenza vaccination, hypertension, obesity and diabetes. Interestingly, three of the genes with risk SNPs found in this study, *RPAIN*, *CIQBP* 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 rs744714 on chromosome 17, were in all high LD, **Figures 2 and 2S**.

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

We also matched cases and controls using the criteria of the same gender and age of  $\pm 5$  years; 68 matched pairs were found. Conditional logistic analysis based on these 68 pairs showed similar estimated ORs as those in our unconditional analysis (**Table 1S**).

## Discussion

Host genetic factors likely 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*, *CIQBP* 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 titers of low-avidity, non-protective anti-influenza antibodies, leading to immune complex deposition and complement activation in the respiratory tract [5]. Of note, one of the genes in which we found a risk SNP, *FCGR2A*, affects handling of immune complexes, and another, *CIQBP*, can activate complement.

The *FCGR2A* gene encodes the Fc $\gamma$  receptor IIA (Fc $\gamma$ RIIA), 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 Fc $\gamma$ RIIA at position 131 (His131Arg). The homozygous His131 genotype (A/A) was significantly enriched 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 Fc $\gamma$ RIIA [15]. The 131His

allele of *FCGR2A* (*FcγRIIA-H131*) has greater affinity than the 131Arg allele (*FcγRIIA-R131*) for all human IgG subclasses [15,16]. The affinity of *FcγRIIA-R131* for IgG<sub>2</sub> is particularly reduced, and *FcγRIIA-H131* is the only human Fcγ receptor that recognizes 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 internalize IgG-opsonized 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 hemorrhagic 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 hypothesize 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 2) was observed with the SNP rs3786054 ( $p= 2.39E-6$ , OR= 3.89), located in the *CIQBP* 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 hypothesize that the risk allele of *CIQBP* 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 therefore can 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'UTR 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 which is involved in RNA trafficking and localization [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 strongly interacts with influenza NEP, and in so doing, hRIP may similarly be a required cellular co-factor for influenza replication [27]. We hypothesize 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. Of note, *CIQBP*, *RPAIN*, and *DHX33* are located in close proximity to each other on 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 *CIQBP* gene than with the SNPs in the *RPAIN* and *DHX33* genes suggests that the *CIQBP* SNP is driving the association between severe pneumonia and this region of chromosome 17, and that the other two SNPs may be acting as neighboring 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 extensively investigated. 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 the 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 those patients with severe pneumonia likely associated with A/H1N1 infection but without viral corroboration. With this stringently defined number of cases, in order to identify more potentially biological important SNPs, we used a significance level of  $1E-4$  to achieve an approximately 80% power. In this context, we can not rule out 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 U.S. 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, Dr. B. Taylor Thompson (Boston) and Dr. Chen Wang (Beijing), 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 *CIQBP* 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 *CIQBP*, 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 with increased immune function, suggesting that this gene may confer risk for severe pneumonia due to increased activation of host immunity.

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## **Author contributions**

Joaquín Zúñiga, David Christiani, Yang Zhao, Gilberto Vargas, Andrew Tager, Annie Pardo and Moisés Selman contributed to study conception and design, SNP data analyses and interpretation, acquisition of data, statistical analyses, obtaining funding and drafting the manuscript. Joaquin Zuñiga, Ivette Buendía, Javier Romo, Diana Torres, Luis Jiménez-Alvarez, Gustavo Ramírez and Alfredo 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 titer measurement and DNA purification. Chau-Chyun Sheu and Li Su performed the genotyping, and Yang Zhao and Feng Chen performed the statistical genetics analyses. Moises Selman and David Christiani had full access to all of the data in the study and take responsibility for the report.

## **Conflicts of interest and financial disclosures**

The authors have not a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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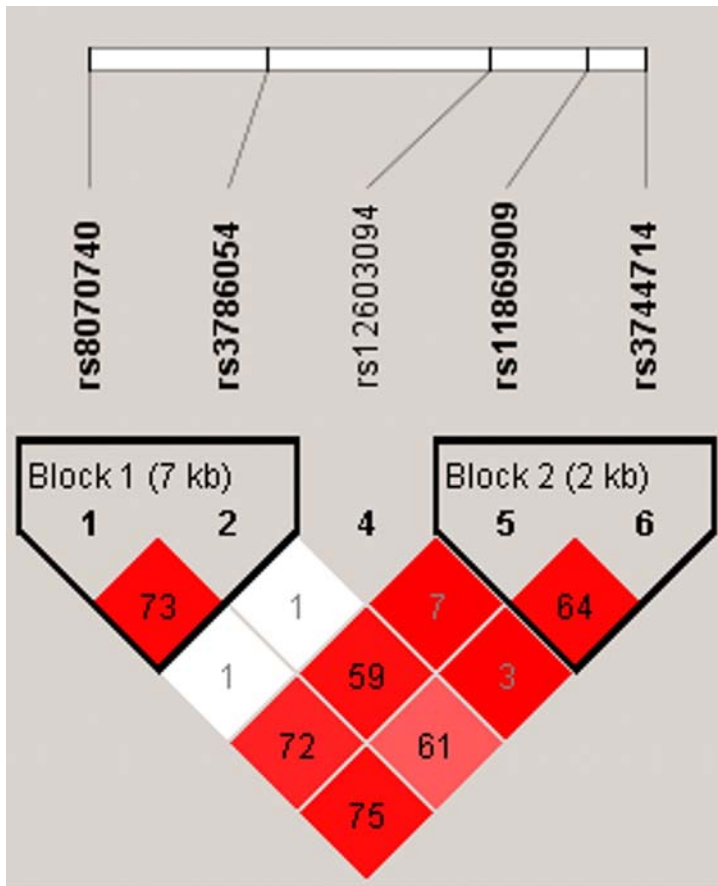
## Figure Legends

**Figure 1:** High resolution CT scan of a patient with severe pneumonia associated to A/H1N1 infection showing multifocal ground-glass attenuation and consolidations and reticular opacities.



**Figure 2:** Linkage disequilibrium (LD) structure of the chromosome 17 region containing the significant SNPs associated with severe pneumonia. The LD plot was generated by Haploview version 4.2. The degree of pairwise LD ( $r^2$ ) is also showed in each block.





**Table 1. Demographic and clinical characteristics of patients with pneumonia associated to the A/H1N1 virus infection.**

<i>Variable</i>	<i>Severe pneumonia patients (N=91)</i>	<i>Household contacts (N=98)</i>	<i>P</i>
<i>Age mean (years) ± SD</i>	38.3±15.6	38.2±15.0	0.9618
<i>Gender male</i>	56(61.54%)	35(35.71%)	0.0005
<i>Obesity</i>	45(51.72%)	42(47.73%)	0.6512
<i>Diabetes mellitus</i>	9(10.00%)	4(4.49%)	0.2488
<i>Arterial Hypertension</i>	14(15.38%)	17(19.10%)	0.5574
<i>Tobacco use</i>	38(41.76%)	20(22.73%)	0.0070
<i>Seasonal flu vaccination</i>	1(1.10%)	23(23.71%)	<0.0001
<i>Health care worker</i>	1(1.10%)	0(0.00%)	0.4815
<i>Mechanical ventilation</i>	42(46.15%)	0(0.00%)	<0.0001
<i>Kirby index (PaO<sub>2</sub>/FiO<sub>2</sub>) mean ± SD</i>	207.6±77.1	-	-
<i>COPD</i>	1(1.10%)	0(0.00%)	1.0000
<i>Asthma</i>	5(5.56%)	1(1.12%)	0.2108
<i>Intestinal Disease</i>	5(5.49%)	1(1.12%)	0.2110
<i>Cerebrovascular disease</i>	3(3.30%)	0(0.00%)	0.2459
<i>Immunosuppression</i>	3(3.30%)	2(2.25%)	1.0000

**Table 2. Result of Single SNP analysis**

SNP	Minor Allele	Chromosome	Location (BP)	Genotype Frequencies*		OR and 95%CI	<i>p</i>	FDR	Gene Name
				Severe Pneumonia	Contacts				
rs1801274	A	1	159746369	33/46/12	13/51/34	2.68 (1.69~4.25)	2.56E-05	0.36	<i>FCGR2A</i>
rs9856661	C	3	54052296	26/48/17	8/51/39	2.62 (1.64~4.18)	5.41E-05	0.51	Unknown
rs8070740	G	17	5272620	17/61/13	10/45/43	2.67 (1.63~4.39)	9.49E-05	0.56	<i>RPAIN</i>
rs3786054	A	17	5279783	16/54/21	5/39/54	3.13 (1.89~5.17)	7.90E-06	0.22	<i>CIQBP</i>

\* 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.

**Table 3. Risk SNPs associated with susceptibility to severe pneumonia ( $p < 0.0001$ ) after multivariable analysis\*.**

<b>Chromosome</b>	<b>SNP</b>	<b>Location(BP)</b>	<b>N</b>	<b>OR and 95% CI</b>	<b><i>p</i></b>
1	rs1801274	159746369	173	3.21(1.93~5.33)	6.45E-6
3	rs9856661	54052296	174	2.59(1.57~4.27)	2.04E-4
17	rs8070740	5272620	174	2.94(1.72~5.02)	7.94E-5
17	rs3786054	5279783	174	3.82(2.19~6.67)	2.39E-6
17	rs3744714	5294801	174	2.92(1.73~4.92)	6.20E-5

\* **Covariates used in the analysis:** obesity, diabetes, arterial hypertension, age, gender and smoking.