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Original Research

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Surfactant Protein B Gene Polymorphism Is Associated With Severe Influenza

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Background: **Surfactant proteins play a key role in alveolar stability. We examined whether single nucleotide polymorphisms (SNPs) related to the surfactant protein genes are associated with severe influenza.**

Methods: **In the first cohort, 12 SNPs related to surfactant protein genes were compared between Chinese patients with severe and mild pandemic 2009 influenza A(H1N1) (A[H1N1]pdm09) infection who were matched for age, sex, and underlying risk conditions. The SNP rs1130866, which was significantly different between the two groups, was further genotyped in a second cohort of patients. Multivariate analysis was performed to control for confounding factors. The genotype frequencies were also compared with those of the general Han Chinese population.**

Results: **This study consisted of 380 patients with A(H1N1)pdm09 infection. In the first cohort of 84 patients, the C allele of rs1130866, an SNP in the surfactant protein B gene (*SFTPB*), was significantly associated with severe disease (OR = 3.37, *P* = .0048), although the *P* value was .057 after Bonferroni correction. In the second cohort of 296 patients, the C/C genotype was confirmed in the univariate analysis to be associated with severe disease. Multivariate analysis of the second cohort showed that genotype C/C was an independent risk factor for severe A(H1N1)pdm09 infection (second cohort: OR = 2.087, *P* = .023). Compared to the general Han Chinese population, the C/C genotype was overrepresented in patients with severe A(H1N1)pdm09 infection (OR = 3.232, *P* = .00000056).**

Conclusions: ***SFTPB*** **polymorphism is associated with severe influenza. The role of *SFTPB* in influenza warrants further studies.** ***CHEST 2014; 145(6):1237–1243***

Abbreviations: A(H1N1)pdm09 = pandemic 2009 influenza A(H1N1); *SFTPB* = surfactant protein B gene; SNP = single nucleotide polymorphism; SP-B = surfactant protein B

There are an estimated 3 million to 5 million severe influenza cases annually worldwide.[1] The avian influenza virus subtypes A(H5N1) and A(H7N9), and the pandemic 2009 influenza A(H1N1) (A[H1N1]pdm09) virus with D222G mutation are particularly virulent.[2-5] Most patients with severe infection have risk factors including extremes of age, pregnancy, and underlying diseases.[6] Obesity was identified as a new risk factor during the 2009 pandemic influenza, and leptin was found to be an important pathogenic mediator in a mouse model of diet-induced obesity.[7,8] A lower IgG2 level was found in patients with severe A(H1N1)pdm09 infection.[9] However, it remains unexplained why many young healthy individuals without these risk factors developed life-threatening illnesses, especially during influenza pandemics.[10-12]

Differences in host genetic background may affect disease outcome. In recent years, single nucleotide polymorphisms (SNPs) have been used to determine genetic predisposition to infectious diseases.[13,14] In our previous study, conducted during the A(H1N1)pdm09 influenza pandemic, we performed a small-scale genomewide association study comparing patients with severe A(H1N1)pdm09 infection and those with mild A(H1N1)pdm09 infection and subsequently identified that genotype T/T of the SNP rs2564978, which is linked to a functional variant of the CD55 promoter, was associated with severe disease.[15] Other genetic

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polymorphisms associated with severe influenza were identified in SNPs related to Fc fragment of IgG, low-affinity IIA receptor; RPA interacting protein; complement component 1, q subcomponent binding protein; toll-like receptor 3; killer-cell Ig-like receptors; and interferon-inducible transmembrane 3.[16-20]

Previous studies have shown the association between polymorphisms in the surfactant protein genes and severe respiratory diseases,[21-23] and surfactant proteins can affect influenza virus replication in vitro.[24,25] Furthermore, surfactant therapy has been used as the salvage therapy in a child with severe A(H1N1)pdm09 infection.[26] In the current study, we used a candidate gene approach to evaluate whether SNPs related to surfactant protein genes are related to severe influenza.

Materials and Methods

Patients

Patients with A(H1N1)pdm09 virus infection were diagnosed between May 2009 and January 2012. Patients were included if their respiratory tract specimens were positive for A(H1N1)pdm09 virus by reverse transcription-polymerase chain reaction or viral culture.[11,27] Clinical information was retrieved from the clinical management system as described previously.[15] Patients were excluded if they were aged < 18 years, were non-Chinese, their archived specimens were not sufficient for testing, or the essential clinical information could not be retrieved from the clinical management system. Patients with severe disease were defined by at least one of the following criteria, as we described previously[15]: required oxygen supplementation, required admission to the ICU, or died. Patients with mild disease were those who did not satisfy the criteria for severe disease. Underlying risk conditions for severe disease were classified according to the definitions of the World Health Organization.[6] The first cohort consisted of patients with severe infection and patients with mild infection, matched by age, sex, and number of underlying risk conditions (Table 1).[6] The second cohort consisted of patients not included in the first cohort but who fulfilled the inclusion criteria. Patients with severe or mild disease in the second cohort were not matched for age, sex, and underlying risk factors. This study was approved by the institutional review board of the University of Hong Kong/Hospital Authority of Hong Kong West Cluster (reference number UW 10-411).

DNA Extraction and Genotyping

Genomic DNA was extracted from archived ethylenediaminetetraacetic acid blood specimens using the QIAamp DNA blood minikit (Qiagen NV) or from respiratory tract specimens using the QIAamp DNA kit (Qiagen NV) as described previously.[9,28] Genotyping was performed as described previously.[15] The first cohort was genotyped using Genome-Wide Human SNP Array 6.0 (Affymetrix Inc). The SNP rs1130866 was genotyped in the second cohort of patients using Sequenom MassARRAY System (Sequenom Inc).

Statistical Analysis

Genetic association was analyzed using PLINK[29] and PASW Statistics 18, release 18.0.0 (IBM). Bonferroni multiple-test correction was performed to correct for multiple testing. Severe cases were compared with mild cases or with the general population using the Han Chinese genotypes in the 1000 Genomes Project sequencing database (Beijing Han Chinese and Southern Han Chinese).[30] The Fisher exact test and Mann-Whitney *U* test were used for the comparison of categorical variables and continuous variables, respectively. To determine whether an SNP was independently associated with severe disease, backward stepwise multivariate regression analysis was used to control for confounding clinical risk conditions. Colinearity diagnostic testing was performed to exclude variables that may be highly correlated. A P value < .05 was considered statistically significant.

Results

A(H1N1)pdm09 Infection

In the first cohort, 42 patients with severe disease were compared with 42 patients with mild disease who were matched for age, sex, and number of risk conditions (Table 1). There was no significant difference between severe and mild cases in the demographics and different underlying conditions. The median age was 48 years with an interquartile range of 39 to 56 years; the male to female ratio was 1:1. Among the 42 severe cases, 31 required positive pressure ventilation (73.8%), 31 required admission to the ICU (73.8%), and 10 died (23.8%). The SNP rs1130866, which is related to the surfactant protein B gene (*SFTPB*), was the only SNP related to surfactant protein that was significantly different between severe and mild cases with a P value < .05 in the allelic χ^2 test (OR = 3.37; 95% CI, 1.404-8.097; P = .0048) (Fig 1A, e-Table 1), although the P value was slightly > .05 after Bonferroni correction (P = .0576). Other nonsignificant SNPs

Manuscript received July 17, 2013; revision accepted November 10, 2013; originally published Online First December 12, 2013.
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Funding/Support: This study is supported by the Health and Medical Research Fund of the Food and Health Bureau of the Hong Kong SAR Government [Project No. 13120842] and the Providence Foundation Limited, in memory of the late Dr Lui Hac Minh.
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DOI: 10.1378/chest.13-1651