Innate immune defect predisposing to severe influenza in a Chinese population

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KEY MESSAGES

1. Genetic polymorphisms in SFTPB and PDE3A genes are independently associated with severe A(H1N1)pdm09 infection.
2. Surfactant protein B has antiviral activity against influenza A(H1N1)pdm09 infection.
3. PDE3A is an antiviral host factor

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Introduction

Influenza virus is a common cause of severe respiratory tract infection. However, most patients with influenza virus infection have only mild respiratory illness. Clinical risk factors for severe influenza include extremes of age, chronic medical illness, pregnancy, and obesity. Patients with severe influenza have been reported to have host genetic defects.[1]

Results from in vitro and animal studies may not be applicable to humans; most genes identified have not been validated in human studies. Moreover, most human studies have comprised mainly Caucasians; the allele frequency differs between ethnic groups, and results from other ethnic groups may not be applicable to the Chinese population. Most human studies did not take into account the differences in comorbidities between patients with severe and mild disease.

This study aimed to use genome-wide association study to identify innate immune defects that predispose Chinese patients to severe influenza virus infection.

Methods

We compared single nucleotide polymorphisms (SNPs) between 42 patients with severe influenza A(H1N1)pdm09 virus infection and 42 patients with mild infection using Genome-Wide Human SNP Array 6.0 (Affymetrix). Both groups were matched for age, sex, and number of risk factors. SNPs were identified for confirmation in another cohort of patients with influenza virus infection using MassARRAY System (Sequenom). Multivariate analysis was performed to control for confounding factors. In vitro studies were performed to confirm the antiviral effects of these genes.

Results

From the genome-wide association study, 30 SNPs were selected for analysis in the second cohort of patients (Table 1), in which the surfactant protein B gene (*SFTPB*) SNP rs1130866 was also significantly associated with severe disease in univariate analysis (odds ratio [OR]=1.928, 95% confidence interval [CI]=1.152-3.227, P=0.012) and multivariate analysis (OR=2.087, 95% CI=1.107-3.934, P=0.023). We then compared the frequency of rs1130866 alleles in patients and in general Han Chinese population (using data from the 1000 Genomes Project). The C allele was significantly associated with severe A(H1N1)pdm09 infection for both recessive pattern (OR=3.232, 95% CI=2.033-5.139, $P=5.6 \times 10^{-7}$) and dominant pattern of inheritance (OR=6.223, 95% CI=1.401-27.640, P=0.006) [Table 2]. We then tested the antiviral activity of surfactant protein B against influenza viruses, as *SFTPB* encodes for surfactant protein B. Plaque reduction assay showed that the IC_{50} of surfactant protein B were 8-24 nM for A(H1N1) and A(H7N9), but the IC^{50} for A(H3N2) and A(H5N1) were >125 nM.

In addition, the phosphodiesterase 3A gene (*PDE3A*) SNPs rs7314545 and rs6487132 were also over-represented (but not significantly [P=0.07]) in severe disease cases of the second cohort of patients. As the level of PDE3A has been shown to be downregulated in the heart of patients with dilated cardiomyopathy or ischaemic heart disease,[2] we re-analysed our second-cohort patients after exclusion of those with heart disease. In this group of patients without heart disease, rs7314545-CT or rs7314545-TT was over-represented in patients with severe disease than in patients with mild disease (17.0% [15/88] vs 8.1% [14/173], P=0.030; OR=2.334, 95% CI=1.071-5.087). Multivariate analysis showed that rs7314545-CT or rs7314545-

TABLE 1 Allelic P values between patients with severe and mild A(H1N1)pdm09 infection

Gene	Single nucleotide polymorphism	Allelic P value
ADIPOR2	rs1044471	>0.05
ADIPOR2	rs11612383	>0.05
ADIPOR2	rs4766415	>0.05
ADIPOR2	rs9300298	>0.05
C4BPA	rs9943077	>0.05
C7	rs3792648	>0.05
C7	rs7713409	>0.05
CARD8	rs2043211	>0.05
CXCL12	rs1801157	>0.05
DSCAM	rs2837657	>0.05
EPHB2	rs4655117	>0.05
HIVEP1	rs2228211	>0.05
HNF4G	rs1805099	>0.05
JAK2	rs10974944	>0.05
KLRK1/ NKG2D	rs4764430	>0.05
MAGI2	rs10279983	>0.05
MAGI3	rs1217228	>0.05
NCAM2	rs2226665	>0.05
NFKBIA	rs3138053	>0.05
PDE3A	rs6487131	>0.05
PDE3A	rs6487132	0.06839
PDE3A	rs7314545	0.06798
PDE4B	rs12029272	>0.05
RNASEL	rs11807829	>0.05
RNASEL	rs11807829	>0.05
SELP	rs2244526	>0.05
SERPINB1(dist=1531), MIR4645(dist=10653)	rs398312	>0.05
SFTPB	rs1130866	0.0204
STAU1	rs6066975	>0.05
TSPAN5	rs1918742	>0.05

TT was an independent risk factor for severe disease (OR=3.447, 95% CI=1.421-8.361, P=0.006). In addition, rs6487132-GG or rs6487132-AG was also overrepresented in patients with severe disease than in patients with mild disease (15.9% [14/88] vs 6.8% [12/165], P=0.019; OR=2.601, 95% CI=1.148-5.897). Multivariate analysis showed that rs7314545-CT or rs7314545-TT and rs6487132-GG or rs6487132-AG were independent risk factors for severe disease (OR=3.447, 95% CI=1.421-8.361, P=0.006).

In vitro viral replication study showed that siRNA-mediated knockdown of PDE3A expression in A549 cells significantly enhanced the replication of A(H1N1)pdm09 virus (Fig).

Discussion

Comparing the genotypes between patients with severe and mild A(H1N1)pdm09 virus infection, we showed that SNPs in the *SFTPB* gene (rs1130866) and *PDE3A* gene (rs6487132 and rs7314545) were associated with disease severity. Multivariate analysis showed that these SNPs were independent risk factors for severe influenza. In vitro studies confirmed that *SFTPB* and *PDE3A* are related to virus replication. Therefore, we successfully identified host factors that play a role in the innate immunity against influenza virus infection.

Surfactant is present in the alveoli, and is important in lowering the surface tension, thereby avoiding the collapse of the alveoli. There are four surfactant proteins, including SP-A, SP-B, SP-C, and SP-D. Previous studies showed that SP-A and SP-D possess antiviral activity against influenza virus. Our study showed that SP-B can also inhibit the replication of influenza viruses.

Phosphodiesterase (PDE) regulates the concentration of the intracellular second messengers cyclic adenosine monophosphate and cyclic guanosine monophosphate, thereby regulating many physiological functions. Regarding virus infections, inhibition of PDE4 by rolipram improved

TABLE 2. Comparison of the rs1130866 genotypes between patients with laboratory-confirmed A(H1N1)pdm09 infection or A(H3N2) infection and in the general Han Chinese population

	CC genotype, No (%)		Odds ratio (95% confidence interval)	P value (Fisher's exact test)
	Different patient groups	General Han Chinese population (n=197)		
Recessive model				
Severe A(H1N1)pdm09 (n=153)	116 (75.8)	97 (49.2)	3.232 (2.033-5.139)	5.60×10^{-7}
All A(H1N1)pdm09 (n=380)	248 (65.3)	97 (49.2)	1.937 (1.365-2.749)	2.37×10^{-4}
Dominant model				
Severe A(H1N1)pdm09 (n=153)	151 (98.7)	182 (92.4)	6.223 (1.401-27.640)	0.006
All A(H1N1)pdm09 (n=380)	369 (97.1)	182 (92.4)	2.765 (1.245-6.141)	0.018

the survival of mice infected with influenza A virus. Polymorphism of PDE8A is associated with HIV-1 replication in primary macrophages. Our study demonstrated that knockdown of *PDE3A* enhanced viral replication, suggesting that PDE3A is an antiviral host factor. We identified that *SFTPB* and *PDE3A* were important susceptibility genes for severe A(H1N1) infection in our population. Together with other susceptibility genes identified to be important for influenza virus infections in humans, we may be able to build a model for predicting prognosis of a patient with influenza virus infection.[1] *SFTPB* and *PDE3A* are involved in the pathogenesis of A(H1N1) pdm09 infection, and additional work on these genes and influenza virus infection may be useful in identifying host- directed antivirals for the treatment of influenza virus infection.

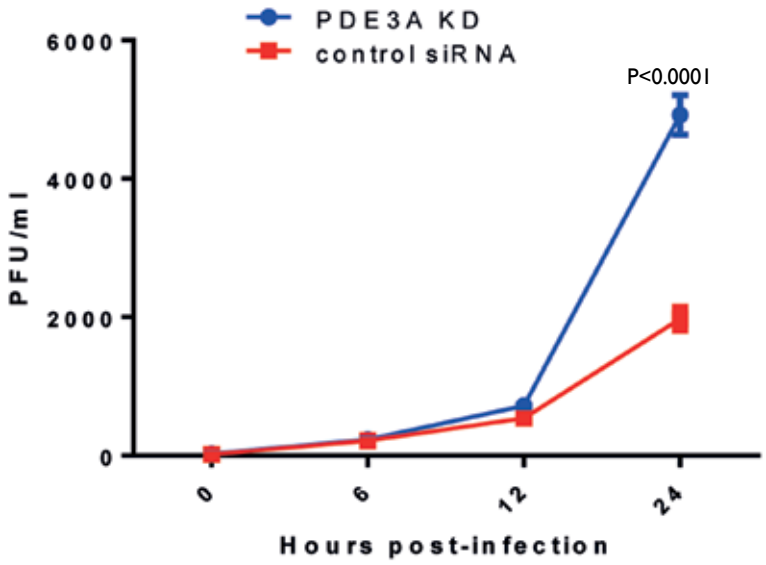

FIG. Effect of PDE3A KD compared with control siRNA on A(H1N1)pdm09 virus replication in A549 cells. A549 cells were transfected with PDE3A-specific siRNA or control siRNA. PDE3A mRNA expression was lowered by >80% at all time points for PDE3A KD cells when compared with cells transfected with control siRNA. A549 cells were infected with A/HK/415742/2009 (H1N1) virus at an MOI of 0.1.

Conclusion

SFTPB and *PDE3A* are independent host susceptibility genes for severe influenza A(H1N1) pdm09 virus infection for the Chinese population in Hong Kong.

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Results from this study have been published in:

(1) To KKW, Zhou J, Song YQ, et al. Surfactant protein B gene polymorphism is associated with severe influenza. Chest 2014;145:1237-43.

(2) To KK, Zhou J, Chan JF, Yuen KY. Host genes and influenza pathogenesis in humans: an emerging paradigm. Curr Opin Virol 2015;14:7-15.

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