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An updated systematic review of the role of host genetics in susceptibility to influenza

Peter Horby,^a Nhu Y. Nguyen,^a Sarah J. Dunstan,^a John Kenneth Baillie^b

^aOxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Vietnam. ^bThe Roslin Institute, University of Edinburgh, Edinburgh, UK.

Correspondence: Peter Horby, Oxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Hanoi, Vietnam. Email: peter.horby@gmail.com

The World Health Organization has identified studies of the role of host genetics on susceptibility to severe influenza as a priority. A systematic review was conducted in June 2011 to summarise the evidence on the role of host genetics in susceptibility to influenza, and this report updates that previously published review. Animal studies suggest that genetic control of susceptibility to severe influenza in mice is complex and not controlled by a single locus, but there is encouraging evidence that some of the host genetic determinants of susceptibility to severe disease may be common across influenza subtypes. Although a number of studies on genetic

susceptibility to influenza in humans have been published recently, all are underpowered and unreplicated, so do not provide robust statistical evidence of an association between the identified genetic loci and susceptibility. One study does however present convincing functional evidence for an important role for IFITM3 in susceptibility to severe influenza in mice, and some evidence that this may also be important in human A/H1N1/pdm2009 infection.

Keywords Animal model, host genetics, human influenza, susceptibility, systematic review.

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Introduction

There is great variability in the severity of disease resulting from infection with influenza viruses, and there are three main determinants of this variability: the intrinsic pathogenicity of the virus, acquired host factors (such as immunity and comorbidity) and intrinsic host susceptibility. Whilst the viral genetic determinants of influenza severity and host immunity have been intensively studied, host genetic determinants are much less well studied.^{1,2} Around one-quarter to one-half of patients with severe pandemic influenza A (H1N1)pdm09 were previously healthy, with no identified coexisting medical condition or other predisposing factors.³ Whilst unrecognised acquired susceptibility factors, such as co-infection, the composition of the upper respiratory tract microbiome or nutritional imbalances (such as vitamin D) may play a role in susceptibility to severe disease in apparently 'healthy' individuals, host genetics may also be important. The study of host genetic factors involved in susceptibility to influenza has the potential to provide a better understanding of the biological pathways leading to severe influenza, which in turn may reveal new therapeutic avenues beyond the few currently available.⁴ In 2009, the World Health Organization identified studies of the role of host genetic factors on susceptibility to severe influenza as a priority.^{5,6} In March 2012, a systematic review summarising the evidence that host genetic factors play a role in human

susceptibility to influenza virus infection or disease was published, and this short report updates that review to include significant new data.¹

Methods

The systematic review was conducted and reported in accordance with the PRISMA guidelines, and the protocol was registered on the international prospective register of systematic reviews (PROSPERO registration number: CRD42011001380. Available at: www.crd.york.ac.uk/prosperto/). The full methods are published elsewhere, and the complete search strategy can be found in the protocol available on the PROSPERO website.¹ Briefly, we conducted a systematic review to summarise relevant published and unpublished evidence of host genetic factors influencing the risk of influenza infection or disease (illness following infection). The search strategy was initially run on 26th June 2011 and was rerun and updated on 11th September 2012.

Results

An additional 149 unique articles published in English since 26th June 2011 were identified, and the titles and abstracts were reviewed. Five were considered relevant and were obtained. A further three relevant articles were identified through a review of the bibliographies of the five selected

papers and personal communications. A total of eight new articles are therefore included in this update. The newly identified publications include one review paper, two animal studies, four human studies and one mixed methods study and are shown in Table 1.

Animal studies

Boivin *et al.*⁷ aimed to identify candidate genomic loci involved in the control of A/HK/1/68-MA20 (H3N2) in 29 recombinant congenic mouse strains using the co-localisation of clinical quantitative trait loci (cQTL) and RNA expression quantitative trait loci (eQTL) to identify genomic areas of interest. The recombinant congenic mouse strains were bred from susceptible (A/J) and resistant (C57BL/6J) mice. The most significant loci identified were *Hc* on chromosome 2, and *Pla2 g7* and *Tnfrsf21* on chromosome 17. The authors found the responses to be sex-specific, with the QTL on chromosome two being associated with susceptibility only in females and the QTL on chromosome 17 only in males. Whilst Boon *et al.*^{8,9} also identified an association with the *Hc* gene on chromosome 2 in their 2009 study of susceptibility to A/H5N1, it was not replicated in their 2011 study. The proteins encoded by the genomic regions identified on chromosome 17 have not previously been associated with susceptibility to influenza.

Bottomly *et al.*¹⁰ examined genetic control of host responses to influenza A/PR/8/34 (H1N1) infection by mapping eQTL. 'High' and 'low' response phenotypes amongst 44 pre-Collaborative Cross mouse strains were identified based on weight loss and viral replication as assessed by immunohistochemical staining of lung tissue at 4 days post-infection. Expression quantitative trait loci mapping was performed on RNA transcripts that were differentially expressed in high and low responders. Twenty-one statistically significant eQTL were identified: 10 that were up regulated in the high responders and 11 up regulated in the low responders. None of these 21 genes have previously been identified as important in influenza A pathogenesis.

Summary of animal studies

Mouse models clearly demonstrate a strong genetic effect on susceptibility to a range of influenza viruses. The *Mx* genes are the best studied, but their relevance to susceptibility in humans is unknown¹ and although the *MxA* gene should be considered a candidate gene for further studies, there are many other candidates. Both the study by Boon *et al.* of H5N1 and that by Boivin *et al.* of H3N2 found a continuum of susceptibility in crossbred strains, suggesting that genetic control of susceptibility to severe influenza in mice is complex and not controlled by a single locus. Bottomly *et al.* also identified a gradient of clinical responses following H1N1 infection of crossbred mouse strains and identified 21

genes that may be involved in genetic control of RNA expression at 4 days post-infection.

Human genetic studies

Four recently published human genetic studies of susceptibility to influenza were identified.

Antonopoulou *et al.*¹¹ investigated the frequency of three SNPs in the tumour necrosis factor gene (*TNF*) in 109 A (H1N1)pdm09 cases and 108 controls. The minor allele (A) at position -238 of *TNF* (SNP rs361525) was more common in cases (frequency = 0.064) compared with controls (frequency = 0.019; $P = 0.016$), and a diagnosis of pneumonia was more common in cases with a least one copy of the minor allele (7/13) compared with cases with no copies of the minor allele (20/96). After correcting for underlying disease in a logistic regression model, the presence of at least one copy of the minor allele in cases was associated with the development of pneumonia with a P -value of 0.045.

The same *TNF* polymorphism (rs361525) was also examined by Ferdinand *et al.*¹², who compared the frequency of eight polymorphisms of two candidate genes (three SNPs in the tumour necrosis factor gene and five SNPs in the mannose-binding lectin gene) in 105 children and young adults with fatal influenza infection compared with population controls. Ferdinand *et al.* found no differences in genotype or allele frequency between case and control groups. The minor allele frequency (MAF) in the 108 controls examined by Antonopoulou *et al.* (0.019) were lower than in the 1000 genomes project (0.05) and the Ferdinand study (MAF cases 0.05), which might explain the significant results. Ferdinand *et al.* did report that fatal influenza cases with methicillin-resistant *Staphylococcus aureus* (MRSA) co-infection had a higher prevalence of a low-producing *MBL2* genotype compared with fatal cases without MRSA co-infection.

Keynan *et al.*¹³ examined the frequency of the chemokine receptor 5 $\Delta 32$ (CCR5 $\Delta 32$) allele (a 32 base pair deletion) in 20 critically ill patients with A(H1N1)pdm09. The CCR5 $\Delta 32$ allele was not found in 10 non-white cases, but 5/9 white cases were heterozygous for CCR5 $\Delta 32$ (MAF = 0.28). The CCR5 $\Delta 32$ allele frequency in white cases was higher than has been reported for healthy Caucasian controls, which is in the range of 10–15%.

Chan *et al.*¹⁴ examined the frequency of immunoglobulin heavy constant gamma 2 (IgHG2) allotypes and Fc gamma receptor 1a (Fc γ RIIa) genotype in 37 severe A(H1N1)pdm09 cases. These two genes are associated with levels of immunoglobulin subclass G2 (IgG2), which has been found to be low in some patients with severe pH1N1 infection.¹⁵ Chan *et al.* reported that IgHG2 allotype was not associated with IgG2 levels in severe A(H1N1)pdm09 cases, and Fc γ RIIa genotype frequencies were not significantly different from that of the general Han Chinese population.

Table 1. Studies of host genetic susceptibility to influenza published between 26th June 2011 and 11th September 2012

Author (Year)	Study/Investigation	Main Reported Findings
Antonopoulou A (2012) ¹¹	Candidate gene case-control association study of the frequency of three SNPs in the tumour necrosis factor gene (<i>TNF</i>) in 109 pH1N1 cases and 108 controls.	The minor allele (A) at position -238 of <i>TNF</i> (SNP rs361525) was more common in cases (frequency = 0.064) compared with controls (frequency = 0.019; <i>P</i> = 0.016). A diagnosis of pneumonia was more common in cases with a least one copy of the minor allele (7/13) compared with cases with no copies of the minor allele (20/96).
Boivin GA (2012) ⁷	Response to infection with a mouse adapted H3N2 (A/HK/1/68-MA20) in 29 recombinant congenic mouse strains created from susceptible (<i>A/J</i>) and resistant (C57BL/6J) mice. Genomic areas of interest identified by co-localisation of clinical quantitative trait loci (cQTL) and RNA expression quantitative trait loci (eQTL).	The most significant loci identified were <i>Hc</i> on chromosome 2, and <i>Pla2 g7</i> and <i>Tnfrsf21</i> on chromosome 17.
Bottomly D (2012) ¹⁰	Animals with high and low response phenotypes following infection with H1N1 (A/PR/8/34) were identified in 44 cross-bred mouse strains. Expression quantitative trait loci mapping was performed on RNA transcripts that were differentially expressed in high and low responders.	Twenty-one genes were identified that may be involved in genetic control of RNA expression at 4 days post-infection.
Chan JF (2011) ¹⁴	Frequency of genetic factors that might affect IgGH2 level by assessing their IgHG2 and Fc gamma receptor IIa (FcγRIIa) genotype in 38 severe pH1N1 cases.	Severe pH1N1 cases had lower levels of IgG2 than mild cases, but IgHG2 allotype was not associated with IgG2 levels and FcγRIIa genotype frequencies did not differ from population controls. The authors concluded that relative IgG2 suppression in this sample is probably the result of cytokine dysregulation rather than genetic factors.
Everitt AR (2012) ¹⁶	<i>In-vitro</i> and mouse studies of the role of interferon-inducible transmembrane protein 3 (IFITM3) in resistance to severe influenza infection. Frequency of <i>Ifitm3</i> single nucleotide polymorphism (rs12252) in 53 people hospitalised with pH1N12009 compared with ethnically matched controls.	Mice lacking a functional <i>Ifitm3</i> gene developed severe viral pneumonia when challenged with normally low-pathogenic viruses, and protection was re-established with reintroduction of IFITM3. <i>Ifitm3</i> SNP rs12252 was found at higher frequency in cases compared with ethnically matched controls.
Ferdinands JM (2011) ¹²	Frequency of 8 SNP's in <i>TNF</i> and <i>MBL</i> genes in 105 children and young adults with fatal influenza compared with population controls.	No differences in genotype or allele frequency between case and control groups. Fatal influenza cases with methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) co-infection had a higher prevalence of a low-producing <i>MBL2</i> genotype compared with fatal cases without MRSA co-infection.
Juno J (2012) ¹⁷	Review of 'immunogenetic' factors associated with severe pH1N1 and avian influenza.	Although the influence of genetic polymorphisms on susceptibility to severe pH1N1 and H5N1 have not been systematically examined, various strands of evidence suggest host immunogenetic variation could play an important role.
Keynan Y (2010) ¹³	A descriptive study of the frequency of the chemokine receptor 5 Δ32 (CCR5Δ32) allele in 20 patients with severe pandemic (H1N1) 2009.	The CCR5Δ32 was not found in 10 non-white cases, and was present in 5/9 white cases. The proportion of white cases with the CCR5Δ32 allele was higher than has been reported for healthy Caucasian controls.

Summary of human genetic studies

Although a number studies on genetic susceptibility to influenza in humans have been published recently, all are underpowered and unreplicated, so do not provide robust statistical evidence of an association between the identified genetic loci and susceptibility.

Mixed methods study

Everitt *et al.*¹⁶ tested the hypothesis that interferon-inducible transmembrane protein 3 (IFITM3) is associated with resistance to severe influenza A infection. Mice lacking a functional *Ifitm3* gene developed severe viral pneumonia

when challenged with a low-pathogenic, murine-adapted H3N2 virus (A/X-31) and an A(H1N1)pdm09 virus (H1N1/09Eng/195), and protection was re-established with reintroduction of IFITM3. An *Ifitm3* single nucleotide polymorphism (rs12252) was found at higher frequency in 53 people hospitalised with A(H1N1)pdm09 compared with ethnically matched controls (minor C allele frequency 0.094 versus 0.028; *P*-value 0.00006). Human lymphoblastoid cell lines that are homozygous for the minor variant (CC) were more susceptible to influenza A/H1N1 (A/WSN/1933) infection and showed decreased levels of IFITM3 expression. When compared to cells expressing wild-type IFITM3, cells

expressing the variant of the IFITM3 protein associated with the minor allele failed to restrict replication of A/H1N1 (A/WSN/1933; A/California/7/2009), A/H3N2 (A/Uruguay/716/2007) and B (B/Brisbane/60/2008). Although the human genetics data alone are insufficiently robust to conclude, there is an association between *Ifitm3* polymorphisms and susceptibility to severe influenza, Everitt *et al.* presented convincing functional evidence for an important role for IFITM3 in susceptibility to severe influenza in mice and some evidence that this may also be important in humans. An effect of IFITM3 has now been observed for influenza A subtypes H3N2, H1N1, H5N1 and influenza B.

Review article

One new review article was identified. Juno *et al.*¹⁷ reviewed a range of immunobiological, gene expression and genetic polymorphism data to explore immunological and genetic factors that may be associated with severe pH1N1 and H5N1 disease.

Discussion

Work summarised here and in the earlier review demonstrates that, in mice at least, the severity of influenza infection is clearly associated with both the pathogen and host genome. The observation that similar patterns of susceptibility or resistance of specific mouse strains are observed for a wide range of influenza viruses suggests that some of the host genetic determinants of susceptibility to severe disease may be common across influenza subtypes. The IFITM3 variant explored by Everitt *et al.* shows an effect across influenza A subtypes H3N2, H1N1 and influenza B. The papers by Boivin *et al.* and Bottomly *et al.* support earlier work showing that susceptibility in mice is polygenic, but the translation of genetic data obtained in mice to humans is challenging. Nevertheless, susceptibility to severe seasonal or pandemic influenza in humans is also likely to be polygenic, as well as being codetermined by pathogen characteristics, prior infection history, comorbidities and environmental factors. Many candidate genes for susceptibility to severe influenza have been proposed, but to date, evidence in humans has only been presented for the *Ifitm3* gene. This gene was selected for investigation based on *a priori* data on the role of IFITM3 in resistance to influenza A, and the majority of evidence for its importance is based on *in-vitro* work. Given the large number of genes that have been identified as playing a potential role in susceptibility to severe influenza in humans, hypothesis-free genome-wide association studies are attractive but are frustrated by the difficulty of recruiting sufficient numbers of well-characterised severe influenza cases to conduct adequately powered and replicable analyses.

The emergence and persistence of a new H1N1 variant (A(H1N1)pdm09) offers a rare opportunity to study genetic

susceptibility to severe influenza in a context that, compared with seasonal influenza strains that have circulated for longer, is less confounded by infection history and pathogen diversity. However, large sample sizes will still be required to detect alleles with a small effect size, and case selection will need to consider confounding by cross-protective immunity, comorbidity and viral heterogeneity. Several groups have compiled series of severe A(H1N1)pdm09 cases, but no single group currently has sufficient cases to conduct an adequately powered genetic study. To have a realistic prospect of identifying susceptibility loci for A(H1N1)pdm09, groups will need to form a consortium, as has been successful for other diseases, to compile case cohorts both retrospectively and prospectively.¹⁸

Conflict of interest

The authors have no potential conflicts to declare.

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