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Calculated Risk: A New SNP Linked to Severe Influenza Disease

Amie J. Eisfeld[1] and **Yoshihiro Kawaoka**[1,2,3]

[1]Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, 53711, USA

[2]Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo, 108-8639, Japan

[3]International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan

Abstract

Clear links between human genes and influenza disease susceptibility are scarce. A recent study uncovered a gene variant coupled to severe influenza and showed how it hampers expression of an antiviral gene key to immune cell survival.

Influenza viruses can cause severe and sometimes fatal disease in humans, owing to differences in the pathogenicity of individual virus strains and variation in human susceptibility. Risk factors—including age, underlying co-morbidities, and pregnancy—influence susceptibility, but do not explain all of the circumstances under which serious influenza-associated complications occur[1]. Host genetic factors have emerged as potential regulators of human influenza disease susceptibility, and may explain severe disease in otherwise apparently healthy individuals. In this issue of *Nature Medicine*, Allen et al. reveal a new single nucleotide polymorphism (SNP) risk allele in the *IFITM3* gene that is strongly associated with severe influenza disease, and further detail a novel mechanism through which this SNP regulates *IFITM3* expression to influence survival of $CD8^+$ T lymphocytes (CTLs) (Figure 1)[2].

IFITM3 is a gene induced by interferons and is a strong candidate for genetic regulation of human influenza susceptibility because of its ability to inhibit influenza virus replication *in vitro* and restrict influenza virus pathogenicity in mice. It also shapes adaptive immune responses to influenza by protecting memory CTL survival during secondary influenza virus challenge[3]. A SNP in the coding region of the *IFITM3* gene (rs12252-C) was previously shown to increase influenza disease severity in some patients[4–8], but the mechanism remains ambiguous.

To identify new *IFITM3*-associated SNPs with the potential to influence influenza disease severity, Allen et al. first assembled candidate SNPs located in the *IFITM3* core promoter and coding regions, and then prioritized those candidates based on common allele frequency

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in European populations and putative functional attributes. A single SNP (rs34481144), located in the *IFITM3* promoter region, satisfied their prioritization criteria. Genotyping analysis of three different patient cohorts revealed statistically significant associations of the rs34481144-A (risk) allele with more severe disease symptoms, earlier virus replication in nasal tissue, and pediatric mortality. Combining data from all three patient cohorts, the authors estimated that individuals carrying the rs34481144-A (risk) allele are ~2.6 times more likely to experience a severe outcome upon influenza virus infection.

Given rs34481144's location in the *IFITM3* core promoter and the rs34481144-A (risk) allele's association with lower *IFITM3* expression in blood cells (based on the authors' query of expression quantitative trait loci data available through the Genotype-Tissue Expression project, www.gtexportal.org), it seems likely that rs34481144 controls influenza disease risk by regulating *IFITM3* expression levels. Allen et al. provide substantial data supporting an association between the rs34481144-A (risk) allele and reduced *IFITM3* expression in immune cells responding to influenza virus infection. They further demonstrate that the rs34481144 genotype directly impacts *IFITM3* promoter function (with the rs34481144-A (risk) allele linked to diminished promoter activity), and identify rs34481144 genotype-specific differences in transcription factor binding to the *IFITM3* promoter. Of particular importance, the rs34481144-A (risk) allele increases binding of the methylation-sensitive CTCF transcriptional repressor, which has not previously been linked to the regulation of *IFITM3* expression.

CTCF promoter binding is disrupted by DNA methylation, and interestingly, the rs34481144-A (risk) allele ablates a CpG methylation site in the *IFITM3* promoter. In their final set of experiments, Allen et al. examined the relationship between the rs34481144 genotype, *IFITM3* promoter methylation, CTCF promoter binding, and *IFITM3* expression in cell types relevant to influenza disease in humans. Their observations are consistent with the notion that the rs34481144 genotype controls *IFITM3* promoter methylation in memory CTLs, and support a model in which methylation at the rs34481144 locus reduces CTCF binding to increase *IFITM3* expression, leading to increased memory CTL survival and more efficient viral clearance from infected airways (Figure 1). Therefore, while a previous study suggested that *IFITM3* expression is important for memory CTL survival during secondary influenza virus challenge[3], the work presented by Allen et al. provides the first evidence that a human allele associated with influenza disease severity directly regulates *IFITM3* expression in this cell type.

The identification of host genetic polymorphisms that regulate risk for severe influenza disease is imperative for reducing influenza-associated morbidity and mortality. Until now, no studies have confidently linked a human genetic variant with influenza disease risk and directly established its mechanism of action. Previously, targeted genetic analyses have associated the *IFITM3* rs12252-C allele with severe influenza disease, particularly in populations with Asian ancestry[5,6], but mechanistic information is lacking. Other attempts at targeted genetic analysis have suggested potential risk factor alleles in the *CCR5*[9], *TNF*[9] and *ST3GAL1*[10] genes, but evidence for association with influenza disease severity is modest. Several groups have attempted genome-wide association studies, and while some SNPs have been identified (e.g., in *CD55*[11] and *FCGR2A*[12]), no candidates have reached genome-wide

significance, none have been authenticated through multiple studies or in multiple patient cohorts, and few attempts to identify mechanisms have been performed. In their work, Allen et al. used a targeted approach to identify a SNP (rs34481144) that regulates the expression of *IFITM3*. The association of the rs34481144-A (risk) allele with higher influenza disease severity across three different patient cohorts, as well as detailed mechanistic studies, provide substantial credibility to their finding, which clearly surpasses the limitations of previous work.

Future studies based on the current work should address several open questions to fully clarify how rs34481144 contributes to human influenza disease. *IFITM3* protein limits influenza virus infection in cell types other than CTL that are central to influenza pathogenicity (e.g., epithelial and endothelial cells), so potential contributions of these cell types to rs34481144-regulated disease severity need to be examined carefully. At the molecular level, mechanistic insights may be garnered by evaluating rs34481144 effects on global *IFITM3* promoter occupancy, since Allen et al. described effects for only a small subset of transcription factors capable of binding *IFITM3* on sites overlapping the rs34481144 locus. Most critically, future efforts must focus on the development of large, well-powered cohort(s) including patients with various human ancestries, both for the wider validation of the rs34481144 allele and identification of additional SNPs that regulate human influenza disease. Ideally, these patient cohorts should include individuals that have been infected with different influenza viruses, since susceptibility may vary depending on the type of influenza virus strain (e.g., seasonal human H1N1 versus avian H5N1 viruses). Moreover, the identification and inclusion of patients that have experienced asymptomatic infections could assist in identification of resistance alleles. Given the time and expense required for the development of such cohort(s), emphasis should also be placed on new animal models that more closely represent human influenza disease (e.g., the Collaborative Cross or Diversity Outbred mice) as a means for identification of potential susceptibility alleles that can be further examined in limited patient cohorts. Ultimately, the identification of genetic risk factors that regulate influenza disease susceptibility may improve clinical case management by assisting in the identification of patients with a greater risk for severe complications, and further, may reveal critical insights into influenza disease pathophysiology. The work reported by Allen et al. is an important step toward achieving this goal.

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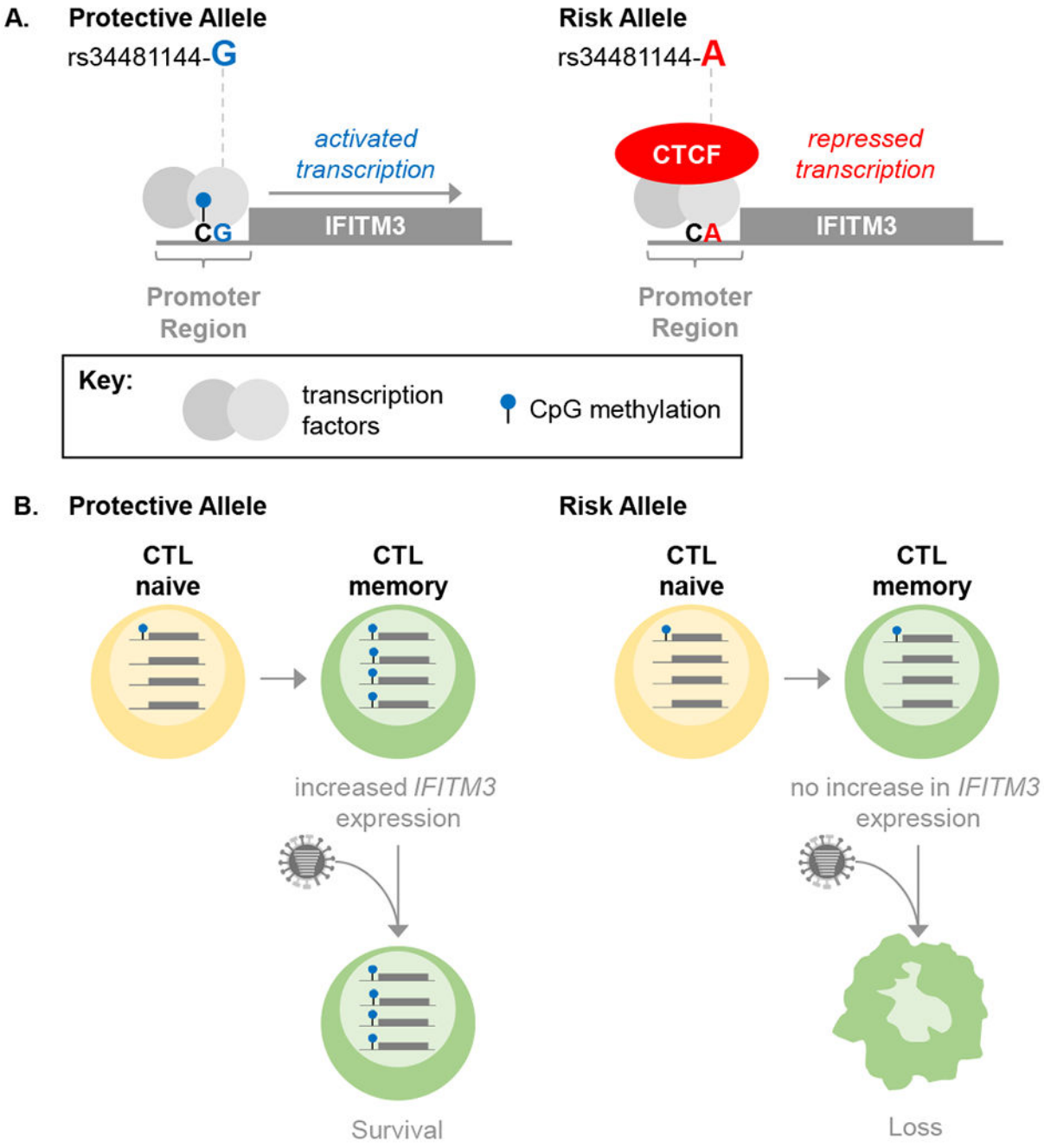

Figure 1. The rs34481144 allele regulates *IFITM3* expression and influenza severity.

(***A***) Shown is the *IFITM3* promoter region and open reading frame. The G (protective) allele promotes methylation of an adjacent nucleotide, while the A (risk) allele ablates methylation. Methylation blocks binding of the CTCF transcriptional repressor, allowing transcription to occur, while in the absence of methylation, CTCF binds to the *IFITM3* promoter and represses *IFITM3* transcription. A key is shown at the bottom of the panel. (***B***)

IFITM3 promoter methylation promotes *IFITM3* protein expression in memory CTL, which protects CTL survival after influenza virus challenge. CTL, $CD8^+$ T lymphocyte.