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The Function and Genetics of the Host IFITM  
Locus

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## **Declaration**

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text. The work here has not been submitted for a degree, diploma, or any other qualification at any other university or institution. I can confirm that this thesis does not exceed the word limit set out by the Degree Committee for the Faculty of Biology.

Sarah Smith.....

Date.....



## Abstract

This thesis focuses on the functional and genetic variation of *interferon-inducible transmembrane protein 3 (IFITM3)* in humans and chickens, and investigates IFITM3's interaction partners during influenza infection. IFITM3 confers resistance to multiple pathogenic viruses, including influenza virus, dengue virus and West Nile virus<sup>1,2</sup>. This has been shown both *in vitro* and in a knock-out *Ifitm3*<sup>-/-</sup> mouse model<sup>3</sup>. Although the current mechanism of restriction is unclear, IFITM3 accumulates within late endosomes and prevents fusion of the virus and host membranes. The disruption of viral pore formation prevents the release of viral nucleic acids and proteins into the cell cytoplasm<sup>4-6</sup>.

These findings were advanced here by analysis of the prevalence of single nucleotide polymorphisms (SNPs) in the *IFITM3* locus of people hospitalised during the 2009 influenza A/H1N1 pandemic. In particular, the rare 'C' allele of SNP rs12252 was identified as being over-represented in hospitalised patients compared to matched controls. Algorithms used by dbSNP suggested that rs12252 caused alternative splicing of the *IFITM3* transcript, potentially creating an N-terminally truncated protein. We hypothesised that the recessive 'C' allele would increase the abundance of truncated proteins with respect to the full-length proteins, explaining the poor response to influenza shown by these patients. Using quantitative RT-PCR, we detected the expression of alternative *IFITM3* transcripts in both primary airway epithelial cells and lymphoblastoid cells. Stimulation by type I interferons increased the abundance of both transcript types. However, no association was found between the rs12252 allele and the ratio of the transcripts.

Thus far, the function of IFITM3 has only been investigated in mammals. Wild birds are an important reservoir for influenza infection, and chickens are particularly susceptible to highly pathogenic strains, such as H5N1. We used the human *IFITM3* transcript to perform BLAST searches on the chicken genome and identified three orthologous IFITM proteins. These proteins were over-expressed in a human cell culture system and were shown to restrict several HA subtypes of influenza virus and two lyssaviruses. Furthermore, endogenous chicken *IFITM3* expressed in DF-1 cells was shown to inhibit influenza A replication.

In order to understand the mechanism used by IFITM3 to restrict enveloped virus entry, co-immunoprecipitations were optimised for various conditions and cell-based signalling assays using luciferase reporter plasmids controlled by an ISRE, an IFN $\beta$  promoter domain or an NF- $\kappa$ B binding domain were carried out. However, IFITM3 was not shown to increase signalling by any of these pathways with or without influenza virus infection.

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## Abbreviations

Full Name	Abbreviation
Acid trehalase-like 1	ATHL1
Acquired immunodeficiency syndrome	AIDS
Beta-1,4-N-acetyl-galactosaminyl transferase 4	B4GALNT4
Bundle-signalling element	BSE
Case fatality rate	CFR
Chicken embryonic fibroblasts	CEFs
Chicken IFITM	chIFITM
Cleavage and polyadenylation specificity factor 30	CPSF30
Colony stimulating factor	CSF
Conserved intracellular loop	CIL
Crimean-Congo haemorrhagic fever virus	CCHFV
C-terminal domain	CTD
Dendritic cells	DCs
Double-stranded RNA	dsRNA
Dulbecco's modified eagle's medium	DMEM
Endoplasmic reticulum	ER
Envelope protein	Env
Epstein-Barr Virus	EBV
Fluorescence lifetime imaging	FLIM
Foetal bovine serum	FBS
Fraction antibody-binding	Fab
Gene Ontology	GO
Genetics of influenza susceptibility in Scotland	GenISIS
Genome wide association study	GWAS
Gravitational acceleration	<i>g</i>
Haemagglutinin	HA
Hepatitis C virus	HCV
High pathogenic avian influenza viruses	HPAIV
Human cytomegalovirus	HCMV
Human IFITM	huIFITM
Human immunodeficiency virus	HIV
Human leukocyte antigen	HLA
IFN stimulated gene factor 3	ISGF3
IFN-gamma activated sequence	GAS
IFN-induced protein with tetratricopeptide repeats	IFIT

IFN-stimulated response elements	ISRE
Infectious bronchitis virus	IBV
Infectious bursal disease virus	IBDV
Influenza A virus	IAV
Interferon	IFN
Interferon-stimulated gene	ISG
Interferon-inducible transmembrane protein	IFITM
Interleukin	IL
Jaagsiekte sheep retrovirus	JSRV
Janus-activated kinase	JAK
Killer-cell immunoglobulin-like receptors	KIRs
Lagos bat virus	LBV
Linkage disequilibrium	LD
Low pathogenic avian influenza viruses	LPAIV
Luciferase	Luc
Lymphoblastoid cell lines	LCLs
Major histocompatibility complex	MHC
Mechanisms of Severe Acute Influenza Consortium	MOSIAC
Melanoma-differentiation-associated gene 5	MDA5
Moloney leukemia virus	MLV
Monocyte-derived macrophages	MDMs
Multiplicity of infection	MOI
Myoxma resistance protein	Mx
Natural killer cells	NK cells
Neuraminidase	NA
Neutrophil extracellular traps	NETs
Newcastle disease virus	NDV
Non-structural protein 1	NS1
Normal goat serum	NGS
N-terminal domain	NTD
Nucleoprotein	NP
Number of non-synonymous substitutions per site	$d_N$
Number of synonymous substitutions per site	$d_S$
Open reading frame	ORF
Oxysterol binding protein	OSBP
Pattern recognition receptors	PRRs
Phosphate buffered saline	PBS
Plaque-forming units	PFU

Polymerase chain reaction	PCR
Primary airway epithelial cells	PAEs
Quantitative reverse-transcription polymerase chain reaction	qRT-PCR
Rabies virus	RABV
Really interesting new gene	RING
Receptor-binding domain	RBD
Relative light unit	RLU
Retinoic acid-inducible gene 1	RIG-I
Revolutions per minute	RPM
Ribonucleoprotein	RNP
Rift valley fever virus	RVFV
RNA-activated protein kinase	PKR
RNA dependent RNA polymerase	RdRp
Respiratory syncytial virus	RSV
Severe acute respiratory infection	SARI
Signal recognition particle	SRP
Signal transducer and activator of transcription	STAT
Simian immunodeficiency virus	SIV
Single nucleotide polymorphism	SNP
Single stranded RNA	ssRNA
SPIa and the RYanodine Receptor	SPRY
Toll-like receptors	TLRs
Tripartite motif-containing protein 5	Trim5 $\alpha$
Tris buffered saline	TBS
Tyrosine kinase 2	Tyk2
Vesicle membrane protein associated protein A	VAPA
Vesicular stomatitis virus	VSV
Viral infectivity factor	Vif
World Health Organisation	WHO
Years of potential life lost	YPLL

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