

Structural Integrity with Functional Plasticity; What Type I Interferon Receptor Polymorphisms Reveal

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Summary sentence

Review of how disease associated polymorphisms that alter the structure of the type I interferon receptors reflect the importance of the receptor structure-function relationship to the maintenance of human health.

Running Title: IFNAR polymorphisms alter receptor function

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Abbreviations

AIDS, acquired immunodeficiency syndrome

C, carboxyl-terminal

Del, deletion mutation

ECD, extracellular domain

ENU, *N*-ethyl-*N*-nitrosourea

Ext, extension mutation

FBN, fibronectin

Fs, frameshift mutation

hCR, helical cytokine receptor

HBV, hepatitis B virus

ICD, intracellular domain

IFN, interferon

IFNAR, interferon alpha/beta receptor

IL, interleukin

LPS, lipopolysaccharide

MS, Multiple sclerosis

N, amino-terminal

PBMC, peripheral blood mononuclear cells

PDB, protein databank

SD, subdomain

SLE, systemic lupus erythematosus

SNP, single nucleotide polymorphism

STAT, signal transducer and activator of transcription

SVR, sustained virological response

TB, tuberculosis

Definitions of unfamiliar terms:

Amino acids are numbered for their position in the mature protein and designated by the three-letter amino acid code throughout.

angstrom – unit of length of interatomic distances; equal to 10^{-10} metres.

binary complex – a protein complex composed of two different proteins.

codon – three nucleotides that form the genetic unit encoding one amino acid.

exon – segment of DNA containing information encoding a protein.

extracellular domain – the region of a cell-bound receptor that is positioned on the cell surface.

frameshift mutation – induces a shift in the reading frame of a transcript during protein translation.

‘hot-spot’ – a residue that makes a major contribution to a binding in an interface.

intracellular domain – the region of a cell-bound receptor that is positioned inside the cell.

intron – a segment of DNA that does not encode proteins and interrupts the sequence of genes.

nonsynonymous mutation – a mutation in a codon that results in a change in the encoded amino acid.

ternary complex – a protein complex composed of three different proteins.

Abstract

The type I interferons (IFNs) activate an array of signalling pathways, which are initiated after IFNs bind their cognate receptors, interferon α/β receptor (IFNAR)1 and IFNAR2. These signals contribute to many aspects of human health including defence against pathogens, cancer immunosurveillance and regulation of inflammation. How these cytokines interact with their receptors influences the quality of these signals. As such, the integrity of receptor structure is pivotal to maintaining human health and the response to immune stimuli.

This review brings together genome wide association studies (GWAS) and clinical reports describing the association of nonsynonymous *IFNAR1* and *IFNAR2* polymorphisms with clinical disease, including altered susceptibility to viral and bacterial pathogens, autoimmune diseases, cancer and adverse reactions to live-attenuated vaccines. We describe the amino acid substitutions or truncations induced by these polymorphisms and, using the knowledge of IFNAR conformational changes, IFNAR-IFN interfaces and overall structure-function relationship of the signalling complexes, we hypothesise the effect of these polymorphisms on receptor structure. That these predicted changes to IFNAR structure are associated with clinical manifestations of human disease, highlights the importance of IFNAR structural integrity to maintaining functional quality of these receptor-mediated responses.

Type I interferons are pivotal to innate immune responses and ultimately, to human health. Understanding the consequences of altered structure on the actions of these clinically significant cell receptors provides important information on the roles of IFNARs in health and disease.

INTRODUCTION

Interferons (IFNs) are innate immune proteins first described in the 1950s (1, 2). They are central to forming an effective immune response with pivotal roles in protection from viral and bacterial infections, in cancer immunosurveillance and in regulating inflammation (3). Structurally all IFNs are α -helical proteins and are broadly classified into three types, the type I (~ 20 subtypes), type II (one member, IFN γ) and type III IFNs (four IFN λ subtypes), distinguishable by the receptors through which they function (4). The type I IFN (T1IFN) family of cytokines is the largest of the three types, including 17 IFN α subtypes, IFN β , IFN ϵ , IFN ω , IFN κ and others, which intriguingly induce differential ligand specific responses despite sharing a heterodimeric receptor complex composed of IFN $\alpha\beta$ receptor (IFNAR)1 and IFNAR2 (5). That the IFNAR receptors can be utilized by multiple ligands possessing as little as ~30% amino acid homology suggests a functional plasticity in ligand engagement by the IFNARs (6). Mutagenesis and biophysical interaction studies, however, provide evidence that structural integrity is also important for efficient ligand binding and functional accuracy (7-9).

The IFNARs are members of the Class II hCR and like all other hCRs fold into well-defined subdomains of ~100 amino acids in length (10). The extracellular domain (ECD) of IFNAR2 is typical of all other Class II hCRs being composed of tandem fibronectin (FBN)-III subdomains (SDs; SD1-2)(11). IFNAR2 exists in three forms; two membrane-bound forms generated by alternative splicing of the *IFNAR2* gene, IFNAR2c which possesses a long intracellular domain that binds Jak1 and STAT1/2s (12, 13) and IFNAR2b which is truncated just inside the membrane and lacks binding sites for known signalling molecules (14). The third form comprises the IFNAR2-ECD but lacks a transmembrane domain and therefore exists as a soluble form (sIFNAR2a) (15). This form of IFNAR2 is found in both

humans and mice and can be generated by either alternative splicing of the *IFNAR2* gene or by ADAM17-mediated proteolytic cleavage at the cell surface (16). In contrast to all other Class II hCRs, the ECD of IFNAR1 possesses a unique four subdomain architecture (SD1-4)(11) which perhaps, reflected by the broad range of affinities it displays for the IFNs (17), aids in the functional plasticity exhibited by the IFNAR complex. IFNAR1 is widely expressed on many different cell types and tissues throughout the body (as cited in (18) (**Fig. 1, Fig 2.**).

The fully functional IFNAR signalling complex requires both IFNAR1 and IFNAR2 to activate the canonical Jak-STAT signalling pathway (19-21), shown to be important for an effective anti-viral response (22). However, collectively, the T1IFNs have also been shown to activate alternative signalling pathways, including the AKT, MAPK and CrkL pathways (**Fig. 1**) (23). Some evidence suggests that IFN β and the IFN α 's may selectively activate certain pathways differentially from each other. IFN β , but not IFN α , reportedly induced the activation of JNK and IFN α , but not IFN β , activated p38 MAPK in a mouse erythroid cell line (24). In hepatocellular carcinoma cells, IFN α 2 and IFN β both activated ERK signalling pathways, but only IFN β showed activation of AKT in these cells (25). IFN β has also been shown to activate AKT *in vivo* via IFNAR1, independently of IFNAR2, suggesting that individual receptors may show situational redundancy with regards to activating alternative IFN-mediated signalling pathways (21).

T1IFN subtypes have a broad range of binding affinities for their receptors (17, 26). In one study, a high relative affinity for IFNAR1 correlated with increased anti-proliferative activity, whereas high affinity for IFNAR2 correlated with greater anti-viral activity (17). In line with this observation, IFN β , which has higher affinity for IFNAR1 than all characterised T1IFNs, was shown to induce a stronger anti-proliferative response than any other T1IFN in

fibrosarcoma cells (17). Conversely, an IFN α variant with high affinity for IFNAR2 but no measurable affinity for IFNAR1 was shown to display good anti-viral activity but no anti-proliferative activity in a range of cell lines (27). Consequently, IFN β and IFN α exhibit differential potencies in certain situations; IFN β is more effective at inhibiting osteoclastogenesis than IFN α 2 (28) and has stronger growth inhibitory effects in some cancer cells than IFN α (29). While evidence suggests that receptor affinity contributes to the differential signalling potencies that IFNs exhibit, other factors, such as the cell type and density of receptors on the cells surface, may also have a role to play in determining cell fate upon IFN treatment (26, 30, 31).

Protein-protein interactions are critical for protein function and depend on the maintenance of the structural features that enable interaction. Factors that alter this structural integrity may influence the way the proteins communicate with binding partners and ultimately change their function. By definition, nonsynonymous mutations of amino acid residues result when a single-nucleotide polymorphism (SNP) occurs in a gene and introduces a missense mutation into the encoded protein; should this occur in a region of a protein required to permit correct protein folding for functionality, the protein may display altered functional characteristics. Monogenic disorders, where a nonsynonymous mutation has altered function of the encoded protein, have detrimental effects on human physiology and the ability to defend against pathogens or respond to an immune stimulus. Nonsynonymous polymorphisms in the ECDs of IFNAR1 and IFNAR2 have been described (see **Table 1**). These SNPs have been identified through genome wide association studies (GWAS), showing significant associations with specific or multiple diseases, or are identified upon genetic screening when patients present with adverse reactions to vaccinations. The nature and location of the residue change and any measured or hypothesized downstream effects on receptor structure and function for each of these nonsynonymous SNPs is discussed in this review. Synonymous

SNPs and those found in intergenic, promoter or intronic regions of *IFNAR1* and *IFNAR2* may also be associated with differential clinical outcomes, however these are discussed elsewhere (32-35).

Given the involvement of the type I IFNs in protective immune responses, polymorphisms that impact the ability of the receptors to fold correctly, alter the strength of the ligand-receptor interaction, or inhibit any necessary conformational change upon ligand engagement, are likely to have an effect on the function of the receptors and therefore the health of a host. Nonsynonymous SNPs have been identified for both *IFNAR1* and *IFNAR2* and are linked to altered health or disease susceptibilities. This review will combine studies on the disease associations of human IFNAR SNPs with current biophysical and structural information about the IFNAR proteins and their functions. We will explore the possible consequences of these polymorphisms on IFNAR protein structure and function, and reciprocally, what these disease association studies and biophysical observations infer about receptor structure and function. These considerations are important for understanding mechanism of action of the receptor system *in vivo* in humans and for the design of therapeutic agonists and antagonists in IFN-deficiency diseases.

NONSYNONYMOUS SNPs IN IFNAR1 AND THEIR DISEASE ASSOCIATIONS

SNP rs2257167 in IFNAR1 is associated with increased susceptibility to a broad spectrum of diseases

Numerous GWAS have demonstrated an association of *IFNAR1* SNP rs2257167 with increased susceptibility to infection by Hepatitis B Virus (HBV) (36-38) and Malaria (39-41), progression of Human Immunodeficiency Virus (HIV) to Acquired Immune Deficiency Syndrome (AIDS) (42) and risk of Multiple sclerosis (MS) (43) and female vitiligo (44). The SNP is also associated with increased risk of death from non-small cell lung cancer (45) and

linked to severe pain in lung cancer patients (46). The clinical manifestations of this SNP appear to support a protective role for IFNAR1 in these multiple and varied diseases. IFNAR1 SNP rs2257167 is located in exon 4 of the *IFNAR1* gene (Chromosome 21:33343393) and converts G to C resulting in mutation of Val141 to Leu on β 3 strand of IFNAR1-SD2, generating the mutant IFNAR1Val141Leu receptor (**Fig. 1. labelled ‘A’; Fig 3A, B; Fig 5; Table 1**). While the level of mutant receptor presented on the surface of patient cells hasn’t been specifically reported, the level of transcription of the *IFNAR1* gene seems to be unaffected by the SNP (43). The native Val141 altered by this SNP is conserved in most species (with the exception of mouse) but is distal to the ligand binding surface on IFNAR1 (21, 47, 48) (**Fig 3A,B**). The crystal structure of unliganded hIFNAR1 suggests that Val141 may interact with neighbouring Trp143, or with residues on adjacent strands of IFNAR1-SD2, including Glu150, Lys174 and Tyr188 (48). However, further studies are required to demonstrate whether the resultant IFNAR1Val141Leu receptor has altered cell surface expression, ligand binding characteristics or downstream signalling capacity.

Homozygous Premature stop codon mutation in *IFNAR1* gene associated with adverse vaccine responses

Following Measles-Mumps-Rubella (MMR) vaccination, a patient presented with symptoms of measles including encephalitis, fever and mild cerebral oedema; tests showed measles virus in blood and cerebral spinal fluid (49). The MMR vaccine comprises live-attenuated viruses for each disease the vaccine protects against, however, tests for rubella and mumps viruses were not reported for this patient (49). The patient was found to be homozygous for a SNP in an essential intron-exon splice site upstream of exon 6 of the *IFNAR1* gene (49). The resulting three alternatively spliced transcripts encoded frameshift (fs) error mutations introducing a premature stop codon at IFNAR1 residue 228 (~60% of transcripts; Val225fs228X) or in-frame deletions resulting in truncated IFNAR1 proteins (30-

40% of transcripts; Val225_Pro232del) (**Fig 1. labelled 'B'; Fig 3A; Table 1**) (49). This SNP introduced premature stop codons in IFNAR1-SD3 and thus any translated protein would likely lack the remainder of the ECD, including half the ligand binding interface and the entire transmembrane and intracellular domains (Fig 1). Compared to cells from control patients, fibroblasts isolated from the patient showed no detectable cellular IFNAR1 and upon stimulation with IFN α 2b or IFN β failed to activate Jak-STAT signalling molecules and to induce canonical Interferon Regulated Genes (IRGs), including *MX1* and *IFIT1* mRNA (49). Whether the transcripts resulted in production of truncated soluble forms of IFNAR1 requires further investigation.

A compound heterozygous mutation in *IFNAR1* gene is associated with adverse vaccine response

Another patient was identified after receiving the live-attenuated Yellow Fever (YF) vaccine and developing symptoms of the disease including fever, hypotension, lethargy and YF viral RNA in blood (49). This patient was found to be compound heterozygous for the intron-exon junction SNP above and another previously unseen mutation in exon 6 of the *IFNAR1* gene. The SNP introduced a nonsynonymous mutation that altered Trp234 to a stop codon on IFNAR1 (IFNAR1Trp234X) (**Fig 1. labelled 'B'; Fig 3A; Table 1**). Similar to the SNP above, any translated protein would lack the remainder of the ECD, the transmembrane and intracellular domains. This patient was also shown to have no detectable cellular IFNAR1 protein and fibroblasts that failed to activate Jak-STAT signalling and IRG expression following IFN treatment (49). Interestingly, prior to this vaccination, this patient had received live-attenuated vaccinations for MMR, poliovirus and Bacillus Calmette-Guerin without adverse side-effects, suggesting that the defect caused by this SNP may be limited to certain vaccines or infectious agents, highlighting the possibility of IFNAR1-dependent and independent signalling pathways contributing to patient response to certain vaccinations. That

this patient reacted poorly to the YF vaccination may suggest an absolute requirement for fully functional IFNAR1 receptor in the response to this vaccine.

SNP rs72552343 reveals a detrimental role for IFNAR1 in *Mycobacterium tuberculosis* infection

IFNAR1 SNP rs72552343 was identified in a population of individuals susceptible to viral hepatitis who also showed increased resistance to *Mycobacterium tuberculosis* infection, an intracellular bacterial pathogen of the lung that causes tuberculosis (TB) (50). The SNP is located in exon 8 of the *IFNAR1* gene (Chromosome 21:33349399-33349404) introducing an in-frame deletion of a single codon in the *IFNAR1* gene resulting in deletion of Pro308 (IFNAR1Pro308 Δ) one of the residues in the strictly conserved di-proline hinge between IFNAR1-SD3 and -SD4 (**Fig 1. labelled 'C'; Fig 3A,C, Table 1**). Paired proline residues located in inter-domain linkers are predicted to be crucial to maintaining local structural rigidity and to prevent unfavourable inter-domain interactions (51). The in-frame deletion of Pro308 is likely to have shortened the distance between IFNAR1-SD3 and -SD4 and therefore altered the ability of these domains to move relative to each other. Despite being distal to the ligand binding interface, the deletion was demonstrated to reduce the affinity of IFN β for the ECD of IFNAR1Pro308 Δ compared to that of normal IFNAR1 (50), confirming that the loss of Pro308 altered the strength of the IFN-IFNAR1 interaction. Furthermore, primary human peripheral blood mononuclear cells (PBMCs) from individuals heterozygous for the SNP demonstrated that the magnitude of IRG induction upon IFN β stimulation was also significantly reduced compared to homozygous normal individuals (50). Despite the mutation being distal to the ligand-binding interface (48), that this mutation alters ligand affinity and IFN β -mediated signalling suggests that the structural integrity of the strictly conserved SD3-SD4 hinge is important for correct functionality of IFNAR1.

This study clearly demonstrated a dichotomy in the role of IFNAR1 in human disease: on the one hand, the altered structural and functional integrity of IFNAR1 demonstrated the host protection this receptor offers from viral hepatitis yet on the other revealed the detrimental effect it has in *Mycobacterium tuberculosis* infection (50). This detrimental role for T1IFNs is in accordance with recent reports that PBMCs from TB patients show a blood IFN signature linked to detrimental clinical outcome in this disease (52) and can suppress TB-protective responses induced by other cytokines (53). Furthermore, IFNAR1 has been shown to contribute to transmission of a non-canonical, IFN β -mediated, proinflammatory signalling pathway (21). While further research is necessary, this pathway may explain why we observe protective or detrimental effects of IFN signalling in different infection settings (54).

SNP in mouse IFNAR1-SD4 leads to susceptibility to viral infection in macrophages

An *N*-ethyl-*N*-nitrosourea (ENU)-generated mouse strain (designated *macro-1*) has been identified with a SNP in exon 9 of the *Ifnar1* gene (Chromosome 21:33349399) (55). This mouse was identified in a screen of *ex vivo* peritoneal macrophages that showed increased susceptibility to infection by a range of viruses (55). The SNP results in an A to C substitution in the *Ifnar1* gene and alters the native Thr315 to Pro in IFNAR1-SD4, (generating IFNAR1Thr315Pro; **Fig 1. labelled 'D'; Fig 3A, D3; Table 1**). That macrophages from the *macro-1* mouse are as susceptible to viral infection as those from STAT1 null mice suggests that the mutation on IFNAR1 in the *macro-1* mouse alters signalling in these mice (55) although the mechanism for the defect is yet to be fully investigated. Using the crystal structure of IFNAR1 in complex with IFN β (21), the native Thr315 mutated in the *macro-1* mouse appears to be involved in main chain hydrogen bond formation with Leu321 and Leu322 around the β 1- β 2 loop of IFNAR1-SD4, likely stabilizing local protein structure (Fig 3D). Models of the structure, by contrast, predict that the substituted Pro restricts the conformation of the residue preceding it (Val314) (56-58) and

due to its ring structure and lack of hydrogen on the α -amino group, this residue is incapable of forming main chain hydrogen bonds with either Leu321 or Leu322 (59). Although the Thr315Pro mutation in the *macro-1* mouse is distal to the ligand binding region of IFNAR1, the mutation is located in a well conserved region on SD4 (**Fig 2.**). By altering the local conformation within IFNAR1-SD4, the Thr315Pro mutation has perhaps altered the way that SD4 associates with the cell membrane, with the membrane-proximal domain of IFNAR2 or permits ligand induced conformational change (60). Further research must be conducted to understand whether the SNP has altered ligand binding affinity of IFNAR1 and the mechanistic effect of the Thr315Pro mutation on IFNAR1 function in the *macro-1* mouse. This ENU-induced SNP appears to support an important role for IFNAR1 in anti-viral defence perhaps through its requirement for activation of STAT1 and the importance of regions of the receptor not directly involved in ligand interaction in transducing a signal.

SNP extends the intracellular domain of IFNAR1 and reduces IFN α -mediated signalling

A young patient presented with fever, anemia, thrombocytopenia, splenomegaly, lymphadenopathy with tests revealing positive blood culture for *Streptococcus viridans*, CMV viremia and *Mycobacterium abscessus* detected in his bone marrow (61). The patient was shown to possess SNPs in both *IFNAR1* and *IFNGR2* genes, related but distinct innate immune receptors (61). The patient had previously received the MMR vaccination with no reported side-effects (61). The SNP in *IFNAR1* gene deleted the terminal stop codon, extending the length of the encoded protein by 46 amino acids (resultant mutant protein is IFNAR1Glu557ext*46X) (**Fig 1. labelled 'E'; Table 1**). Interestingly, while the level of IFNAR1 on the surface of fibroblasts was similar to those of control patients, these cells showed impaired IFN α -mediated STAT1 and STAT2 phosphorylation, STAT1 nuclear translocation, IRG expression and could not mount an effective IFN α -mediated response to CMV viremia (61). Collectively, these observations suggest that, although IFNAR1 was

present on the surface of the patients' cells, the function of the receptor was significantly impaired. The potential structural change that this SNP induced on IFNAR1 highlights what very little we know about the mechanism of action of the intracellular domain (ICD) of this receptor. It has previously been hypothesised that to effect STAT1/STAT2 cross-phosphorylation, the ICD of IFNAR2 may loop around the carboxyl terminal of the shorter IFNAR1-ICD enabling canonical Jak-STAT signalling (19). Although no experimental evidence has been shown to support this hypothesis, an extension of 46 amino acids on IFNAR1-ICD may reduce the likelihood of its occurrence thereby reducing signalling via the canonical Jak-STAT signalling pathway. Alternatively, to induce a reduction in IFN α -mediated signalling, the extension to IFNAR1-ICD may instead stabilise recruitment of intracellular negative regulators to the ternary signalling complex thereby reducing signal transduction. With regards to this patient however, it must be pointed out that this individual also possessed a SNP in the *IFNGR2* gene which induced a frameshift error in the receptor resulting in reduced levels of IFNGR2 and abrogated IFN γ -mediated signalling (61). Thus, interpretation of the connection of this patient to the displayed phenotype is complex due to mutation of both type I and type II IFN receptors.

NONSYNONYMOUS SNPs IN IFNAR2 AND THEIR DISEASE ASSOCIATIONS

Polymorphisms in IFNAR2 signal peptide increase disease risk associations

IFNAR2 SNP rs2229207 and IFNAR2 SNP rs1051393 are both located in exon 1 of the *IFNAR2* gene (Chromosome 21:33241945 and Chromosome 21:33241950, respectively) and both induce nonsynonymous mutations within the signal peptide of IFNAR2 pro-protein (**Fig 1. labelled 'F'**). SNP rs2229207 is associated with an increased risk of a sustained virological response (SVR) to hepatitis C (62), while SNP rs1051393 is associated with an increased risk of multiple sclerosis (43), hepatitis B infection (63), hepatitis C chronicity (64) and with severe pain in lung cancer patients (46) (**Table 1**). Both SNPs induce changes from large

hydrophobic Phe residues to smaller Ser and Val residues, respectively, in a region of the signal peptide known for being enriched in hydrophobic residues (65). Similar mutations in signal sequences of other proteins have been shown to alter nuclear export of the mRNA (66), disrupt interactions of the mRNA with translational machinery thereby targeting the mRNA for degradation (67) or alter trafficking efficacy of the pro-protein (65). In the case of the two SNPs in the *IFNAR2* signal peptide above, any of these scenarios would affect the levels of IFNAR2 that is translated and therefore secreted from the cell, either as the soluble form or for cell bound forms. Experimental evidence for the effect of these mutations on IFNAR2 protein levels is yet to be demonstrated.

SNP rs775739391 reveals the necessity of IFNAR2 in response to live-attenuated viral vaccination

Following an adverse reaction to inoculation with the live-attenuated MMR vaccine, a patient was shown to possess a novel nonsynonymous SNP in the *IFNAR2* gene (68). SNP rs775739391 leads to deletion of a single nucleotide in exon 5 of the *IFNAR2* gene (Chromosome 21:33246807) within the codon for Glu77, inducing a frameshift error and the generation of a premature stop codon (**Fig 1. labelled 'G'; Table 1**) (68). The subsequent protein would be expected to be truncated six residues after His76 and thereby lacking the rest of SD1 and half the ligand binding interface, all of SD2, the transmembrane and intracellular domains and thereby likely not be found on the cell surface (mutated protein referred to as IFNAR2Glu77fs83X, Table 1). Indeed, in fibroblasts derived from this patient IFNAR2 protein could not be detected by Western blot (68) and subsequent IFN treatment of these cells failed to induce activation of canonical downstream signalling components, such as Jak1 and STAT1 (68). The infant succumbed to encephalitis as a result of uncontrolled replication of the live-attenuated vaccine viruses, demonstrating the requirement for IFNAR2 to respond effectively to this vaccination.

A pro-inflammatory IFNAR2-independent signalling axis mediated via the high affinity IFN β -IFNAR1 interaction has been identified that contributes to lethality in a mouse model of sepsis (21). While a high affinity interaction is also conserved in humans between IFN β and IFNAR1 (17), no direct evidence for the existence of an IFN-mediated, IFNAR2-independent pathway has yet been specifically identified in the human system. Targeted knock out of IFNAR2 in an immortal human cancer cell line has demonstrated that if signalling can be transmitted through a single human IFNAR receptor, it is likely to show cell- or IFN-subtype specificity since these cells failed to induce any measurable IFN-mediated signalling, canonical or non-canonical (69). However, as patients deficient in functional IFNAR2 receptor are identified, it may be increasingly possible to access a suite of primary cells to investigate if such signalling defects are indeed cell-specific and result from a lack of the protective effects demonstrated by IFNAR2 or from an undetected proinflammatory pathway mediated via the remaining IFNAR1 receptor.

ROLE OF IFNARS IN HUMAN DISEASE

Due to the limitations of human clinical studies, mouse models have been critical in understanding the mechanism of action attributable to a single protein and its involvement in disease pathogenesis or suppression (22, 70-72). It is through these studies that the dichotomous nature of IFNAR-mediated functions against viral versus bacterial pathogens has been observed. These models have demonstrated that IFNAR1 is protective against most infections by viruses and extracellular bacteria (73) and suppress disease in certain non-infectious diseases such as pulmonary hypertension (74), multiple sclerosis (75) and in maintenance of intestinal health (76). In contrast, IFNAR1 has been shown to be detrimental to the host during infection by certain intracellular bacteria (54, 73) including *Listeria monocytogenes* (77), *Salmonella enterica* serovar *Typhimurium* (78) and *Mycobacterium tuberculosis* (79). A similar detrimental role for IFNAR1 during *Plasmodium* infection has

also been demonstrated whereby IFNAR1-mediated signalling impedes activation of the adaptive immune response (80). One intriguing possibility is that IFNAR1 may be selectively protective against some bacterial pathogens but detrimental against others based on whether infection is intra- or extra-cellular in nature (81). IFNAR1 also has a detrimental role during certain autoinflammatory diseases including in Sjogrens syndrome (82), in neuropathic pain (83) and systematic lupus erythematosus (SLE). Both *Ifnar1* deficiency and the use of an IFNAR1 neutralising antibody have demonstrated that IFNAR1-mediated signals are detrimental in mouse models of SLE (84, 85). Importantly, this observation has been translated into the clinic with an anti-IFNAR1 neutralising antibody showing therapeutic potential in humans for the treatment of SLE (86-89) demonstrating the translatability of mouse studies to humans in the type I IFN system in this disease.

IFNAR2 is critical for host protective responses to influenza infection (90) and likely many more viruses due to the fact that this receptor is the primary binding partner for IFN ligands and since it recruits STAT1 and STAT2 to the receptor complex (13), molecules essential for an effective anti-viral response (71). The soluble form of IFNAR2 (sIFNAR2) is found widely in normal human body fluids (91) and has been implicated in a number of autoimmune diseases (92, 93). Reduced levels of sIFNAR2 in the serum of peripheral blood has been identified as a prognostic marker in naïve multiple sclerosis (MS) patients, with circulating levels increasing in MS patients undergoing IFN β treatment (92). As soluble IFNAR2 can bind IFNs directly to potentiate a signal (15), that soluble IFNAR2 is depleted in naïve MS patients suggests that it may be binding circulating IFN to mediate a signalling response, thereby reducing its own levels in serum. On the other hand, active MS lesions have increased levels of ADAM17 (94), the protease shown to generate soluble IFNAR2 by proteolytic cleavage (16). Thus, during active MS and IFN β therapy, increased ADAM17 may promote a rise in circulating soluble IFNAR2 levels and may therefore be important in a

positive response to IFN β therapy. In a mouse model of SLE, sIFNAR2 has been found to contribute to the production of auto-antibodies (93) suggesting an important role for this receptor in the pathogenicity of this and perhaps other autoimmune diseases. Although the mechanism is yet to be fully elucidated, sIFNAR2 has been shown to function via a trans-signalling mechanism, akin to the soluble IL-6 receptor (95), whereby it can regulate IFN signalling (15, 91, 96, 97).

Structure of IFNAR1-ECD

To date, three structures of IFNAR1-IFN complexes are available; two human protein complexes and one from mouse. The human IFNAR1-IFN complexes demonstrate the binding of two different ligands – one with a hIFN α 2 variant (IFN α 2-YNS; PDB accession codes 3SE3) and one with IFN ω (PDB accession code 3SE4 (48)). The third structure is a murine complex with IFN β (PDB accession code 3WCY (21)). All complexes show an overall conservation of the general recognition of IFN by IFNAR1, with SD1-3 forming a continuous and extensive interface – IFNAR1-SD1 is centred on the D helix^{IFN}, SD2 on the B helix^{IFN} and SD3 on the C helix^{IFN} of the ligands. Due to the four-domain architecture of IFNAR1, this receptor forms “elbow” contacts to IFN between the IFNAR1 SD1-2 and contacts distal to the “elbow” between IFNAR1 SD2-3. IFNAR1 SD4 is extended from the primary site of IFN recognition and makes limited contacts via its β 2- β 3 loop (**Fig 4**). IFNAR1 SD1-3 each contribute approximately one-third of the binding interface (**Fig 4**). By contrast, IFNAR1-SD4, which was resolved only in the murine IFN β -IFNAR1 complex (21), is offset by $\sim 90^\circ$ from the planar arrangement of IFNAR1 SD1-3 and makes limited contact with IFN β (**Fig 4**, **Fig 5**). The interface on IFNAR1-SD4 may be specific to IFN β or specific to the murine system, since a comparable crystal structure with human IFN β is yet to be determined. The majority contacts with IFNAR1 SD1-3 is consistent with biochemical analyses that show this region is sufficient for *in vitro* ligand binding whilst SD4 is

dispensable (21, 60, 98, 99)(**Fig 2.**). That three FBN-III domains are required to form the ligand binding region on IFNAR1 again demonstrates the unique mode in which IFNAR1 binds its ligands, a mechanism unmatched in the Class II hCR family of receptors. While IFNAR1 SD4 may not play a major role in direct ligand binding, it seems to play an important role in signal transduction (98).

Residues in the ligand-IFNAR1 interface

'Hot-spot' residues: As determined by site-directed mutagenesis of interaction interfaces, individual residues that make major contributions to the overall affinity of an interaction are referred to as 'hot spot' residues. Despite the low affinity of the IFNAR1-IFN α interaction, integrity of this interface is important for full IFN α activity, since its interruption removes IFN α -induced anti-proliferative activity (100). In this interface, only Tyr70^{AR1} (IFNAR1 SD1) and Phe238^{AR1} (IFNAR1 SD3) are described as 'hot-spot' residues with minor contributions from other residues (**Table 2**) (48). In contrast, the IFNAR1-IFN β interface is much more extensive (**Fig 5.**) with major contributions found on the three membrane-distal SDs and a minor contribution from residues on IFNAR1 SD4 (Table 2) (21). On IFNAR1 SD1, Tyr70 and Tyr98 make major contributions to binding IFN β , the former a 'hot-spot' residue conserved with IFN α and IFN ω (**Fig 2.**). On SD3, two prominent residues, Tyr240^{AR1} (the homologous Phe238 in human IFNAR1 is also a 'hot-spot' residue in the interface with IFN α and IFN ω) and the unique Tyr274^{AR1} form a 'hot-spot' of interaction in the IFNAR1-IFN β interface; these residues are also important in determining the magnitude of the signalling response and biological activities exhibited by IFN β (99). Residues that make minor contributions to the ligand interface are also found on other regions of SD1-3^{AR1} (**Fig 2.**).

Ligand-specific residues: Comparison of the IFNAR1-IFN interfaces in the three published crystal structures (21, 48) reveals that specific residues on IFNAR1 contribute

preferentially to binding different ligands. For IFNAR1, the ability to associate with the large array of type I IFNs, often with low sequence identity, is likely achieved by the “moulding” of the receptor around a structurally conserved core or common ‘anchor points’ on the cytokines (48). Consistent with this hypothesis, the murine IFNAR1 orientation when bound to IFN β deviates by 16° from the human IFNAR1 bound to IFN α 2-YNS or IFN ω , yet retains common sites of recognition (21) (**Fig 4, Fig 6.**). The variation observed in the affinity of IFNAR1 for different IFN subtypes (17, 26) is possibly accounted for by differences in the docking angle and hence the interacting surface area of IFNAR1. Namely, IFNAR1 buried a surface area of 3300 Å² on IFN β compared with 2030 Å² and 2200 Å² for IFN α -YNS and IFN ω , respectively (**Fig 6.**). The extent of this interaction area correlates with the ~100-fold affinity difference between IFN β and IFN α 2 for IFNAR1. Notwithstanding these differences, common sites for recognition or potential ‘anchor points’ are present for IFNAR1 SD1-3 that are conserved across species (21, 48). Interestingly, Tyr274^{AR1} located in the mIFNAR1-IFN β interface on SD3^{AR1} is important for formation of this high affinity interaction and mIFN β -mediated signals (99). Since this residue is not conserved across species, and the residue with which it structurally aligns (Gln272^{AR1}) was not identified in the hIFNAR1-hIFN interfaces (48), Tyr274^{AR1} may be an IFN β -specific anchor point on IFNAR1 or it may contribute to the species specificity exhibited by most IFNs. Thus, whilst there is considerable scope for “sliding” of IFNAR1 to accommodate various IFN subtypes binding at different affinities, there may nonetheless be key points of ligand-specific recognition on IFNAR1 SD1-3.

The SNP identified by Hernandez et al (49) which converts Trp234 to a premature stop codon would be predicted to produce a truncated, soluble receptor comprising IFNAR1 SD1-2 and small portion of SD3 (**Fig 2.**). This protein would likely possess the ligand binding ‘hot-spot’ residues on SD1 (Tyr70) however it would lack the ‘hot-spot’ and ligand-specific

residues on SD3 that make significant contributions to the strength of the IFNAR1-ligand binding interface (including Phe238)(48, 99). This truncated form of IFNAR1 may therefore be unable to form a stable interaction with any ligand. Of the other SNPs above, none of the mutated residues have been implicated directly in the ligand binding interface. However, given that the SNPs show associations with diseases, the structural effect of these mutation on IFNAR1 is likely altering another characteristic of the receptor.

Alterations to inter-domain flexibility or mobility may alter receptor functionality

The function of cytokine receptors can also be dependent on the ability of the receptor to undertake conformational change(s) upon ligand binding to initiate secondary interactions or signal transduction (101, 102). Polymorphisms that restrict the ability of a receptor to undergo any conformational change necessary for optimal ligand binding or signal transduction could therefore alter receptor function. Helical cytokine receptors, including IFNAR1 and IFNAR2, possess highly conserved di-proline motifs in the hinge regions between subdomains, giving structural rigidity to receptors and regulating domain-domain flexibility (11, 51). Disruption of these motifs by mutation or substitution are known to lead to altered domain-domain flexibility in cytokine receptors (103, 104). For IFNAR1, SNP rs72552343 causes deletion of Pro308, one of the two highly conserved residues in the IFNAR1-SD3/SD4 di-proline hinge (50). Despite the fact that Pro308 is not directly involved in the ligand binding interface on IFNAR1, the resultant receptor showed reduced affinity for IFN β and reduced IRG induction (50). Deletion of this residue on IFNAR1 likely alters the kinetics of the ligand-receptor interaction indirectly via a mechanism that likely affects inter-domain flexibility and therefore the stability and life-time of the ternary signalling complex (105).

Upon ligand binding, IFNAR1 undergoes a loss in mechanical stability, manifested as cooperative movements between IFNAR1-SD2 and SD3 (106, 107) and a multi-step

conformational change that is propagated along the receptor (60). Both the loss in mechanical stability and the conformational change are required for effective signal transduction (60, 106, 107). Together these observations suggest that all four subdomains of IFNAR1 are required to generate the cooperative movements necessary for effective signal transduction via this receptor. Given this hypothesis, it is likely that any force that perturbs these interdomain movements may alter receptor functionality. Indeed, an antibody showing clinical utility for the treatment of scleroderma (108) and SLE (109) has been shown to neutralize IFNAR1 signalling by binding Arg279 on IFNAR1-SD3 (110), a residue not implicated directly in the ligand binding domain for IFN α or IFN ω (48). While the antibody blocks IFN-mediated STAT activation and IRG induction (88), its mechanism of action may involve steric hinderance of ligand binding to IFNAR1 or the propagation of the conformational change essential for ligand-induced signal transduction (60). Likewise, although the mutation caused by IFNAR1 SNP rs2257167 induces a mutation distal to the ligand binding domain, given the location of the Val141Leu substitution adjacent to the SD2-SD3 hinge region, the mutation may alter the relative interdomain movements, thereby altering receptor function. Experimental evidence to these hypotheses is required.

While the relative affinity of ligands for the IFNARs has led to the hypothesis that IFNs bind initially to IFNAR2 with high affinity, followed by recruitment of IFNAR1 (the low affinity receptor)(98), we and others have hypothesized a potential third step that takes into account the mobility of IFNAR1-SD4 in relation to the three membrane distal domains (21, 111). Following IFNAR1-SD1-3 binding to ligand, we propose that there is a conformational movement of the mobile IFNAR1-SD4 that contributes to formation of a functional signalling complex (**Fig 3A**). This aligns with a study that used Förster resonance energy transfer spectroscopy and single-particle electron microscopy to demonstrate that ligand binding induces a conformational change in IFNAR1 that positions SD4 in close proximity to that of

IFNAR2 SD2 (60). Although a ligand-independent association between IFNAR1 and IFNAR2 has not been reported, this hypothesis may explain why IFNAR1 SD4 cannot be recapitulated by substitution with membrane proximal domains from homologous Class II hCRs, including those of IFN λ and IL10 (98).

Structure of IFNAR2-ECD

Protein crystallographic studies of the hIFN α 2-YNS and IFN ω ternary complexes and IFNAR2-IFN α 2-YNS binary complex (48) provide a consensus on the IFNAR2 binding interface that is composed of primary sites on the AB-loop^{IFN} and the A and E helices^{IFN} (**Fig 6**). Together, these sites form parallel hydrophobic and hydrophilic striations involved in IFN α 2 binding and indicate that there is an inherent flexibility of the IFNAR2-ECD binding surface, which allows it to accommodate various type I IFNs (112). Unlike other Class II hCRs that typically utilise both SD1 and SD2 in recognition of their ligands, IFNAR2 binds predominantly (~97 %) via SD1 (**Fig 6**). This interface is extensive (~1800 Å²) and is comparable in size to the IFNAR1-SD1-3 interface with IFN α and IFN ω (2030 Å² and 2200 Å², respectively) (48). Early mutagenesis studies indicated that IFN α 2 and IFN β bind at a similar location on IFNAR2, but with a different distribution of contributions from ligand-specific residues (**Table 1**) (9, 17, 113). Primarily for IFN α 2, Met46^{AR2} is the ‘hot-spot’ residue in the interaction, while numerous distal and proximal residues also contribute to the interface (see **Table 1**)(48). For the IFNAR2-IFN β interface no ‘hot-spot’ residue has been identified, however His76^{AR2} and Trp100^{AR2} are important for binding, residues shared by the IFNAR2-IFN α interface (see **Table 1**) (9, 48, 113, 114). Asp98^{AR2} appears to be unique to the IFNAR2-IFN β interface in comparison with the IFN α interface (114). The SNP rs775739391 which generates IFNAR2Glu77fs83X thereby introducing a premature stop codon in IFNAR2 SD1, is predicted to encode a protein lacking numerous residues from both the IFN α and IFN β interfaces, including Asp98^{AR2} and Trp100^{AR2} (48, 114).

Structure of the Ternary IFNAR1-IFN-IFNAR2 Complex

The IFNAR1-SD4 and IFNAR2-SD2 are relatively untethered to the ligand and so are afforded degrees of freedom in movement (**Fig 4a. IFNAR1, Fig 5. AR2only**). This is especially the case for IFNAR1-SD4 that is displaced from the ligand-binding subdomains IFNAR1 SD1-3 by a long unstructured linker (**Fig 4**). Indeed, in electron microscopy images of a hIFNAR1-hIFN α 2-hIFNAR2 ternary complex, IFNAR1-SD4 was shown to exist in variable locations in relation to IFNAR1-SD1-3 (111). This study even suggested that IFNAR1 and IFNAR2 may interact directly upon ligand binding (111). Similar structures were shown in electron microscopy images of the hIFNAR1-hIFN β -hIFNAR2 ternary complex (60). IFNAR1-SD4 was not resolved in the crystal structures of the ternary complexes with IFN α 2-YNS and IFN ω but has been previously modelled in an orientation similar to the planar arrangement seen for the IFNAR1 SD1-SD2 (115). Such an arrangement is not observed in the mIFNAR1-mIFN β binary structure in which IFNAR1-SD4 sits orthogonal to the IFNAR1-SD1-3 (**Fig 4**) (21). This orthogonal arrangement is the more likely one for IFNAR1-SD4 as it aligns with IFNAR2-SD2, which similarly sits orthogonally to the IFNAR1-SD1-3. Thus, as a ternary complex, the membrane proximal domains of IFNAR1 and IFNAR2 may be brought into close proximity with the transmembrane domains close to parallel (**Fig 3A**). Whether there is a direct interaction between IFNAR1-SD4 and IFNAR2-SD2 is a matter of debate.

Role of soluble IFNAR2 in IFNAR structure and function

In both human and murine systems, sIFNAR2 is produced through alternative splicing of the *IFNAR2* gene (116) or by ADAM17-mediated proteolytic cleavage (16). Human sIFNAR2a differs from hIFNAR2c-ECD by the substitution of the C-terminal Ser212Ala213 by Phe212Ser213 (117). In contrast, mouse sIFNAR2a differs from mIFNAR2c-ECD by an additional 11 amino acid residues at the C-terminal tail (118). Human sIFNAR2a is found in

various fluids, including serum, urine, and cerebrospinal fluid (91, 92, 119). In mouse, the transcript for sIFNAR2a is found in most tissues, and is more abundantly expressed than that encoding the full-length transmembrane form, mIFNAR2c (15). The biological importance of sIFNAR2a is implicated by findings that link elevated serum sIFNAR2a to human viral infections including hepatitis C and HIV, to chronic inflammatory disorders such as SLE and to various cancers (92, 120-125). Similarly, mice engineered to express elevated sIFNAR2a, thereby mimicking the elevated sIFNAR2a seen in human diseases, demonstrate increased susceptibility to lipopolysaccharide (LPS)-mediated septic shock (97). In contrast, the level of detectable serum sIFNAR2a was demonstrated to be significantly reduced in naïve multiple sclerosis (MS) patients and that IFN β treatment increased the level of the soluble receptor (92, 125). A mouse model of multiple sclerosis also demonstrated that sIFNAR2a can potentiate IFN β signalling and may have intrinsic properties that inhibit T-cell proliferation, contributing to improved clinical outcomes (126). Regardless of these studies, the mechanism of action of sIFNAR2a in health and disease remains to be fully elucidated.

Concluding remarks

Single nucleotide changes in the genetic code that alter the structure of the IFN receptors can have measurable and profound effects on the function of these proteins, influencing an individual's risk of disease and the quality of the immune responses they mount. Interferons are potent cytokines inducing highly regulated biological responses that protect from infections and cancer and maintain physiological homeostasis. GWAS studies and case reports of individuals with SNPs who display aberrant responses to otherwise safe, live-attenuated vaccinations have given us an increased understanding of the importance of IFNAR structure to maintaining health and initiating appropriate immune responses.

Using known structural information on the receptors, we have discussed the predicted structural effects of SNPs on the IFNARs and the evident or possible downstream

consequences of these changes on receptor function, providing insight into the physiological roles of the IFNARs. Increased understanding of the influence IFNAR structural features have on normal receptor function will better inform our knowledge of the roles of these receptors in maintenance of health and defence against disease.

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Figure Legends

Figure 1A

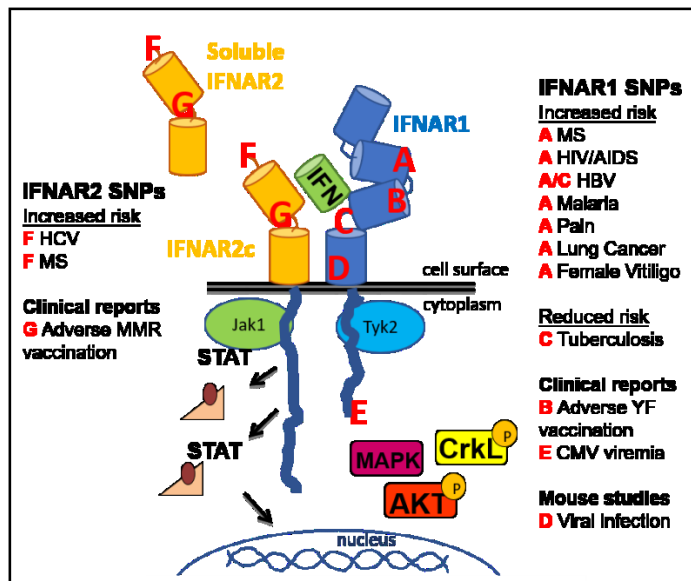


Figure 1B

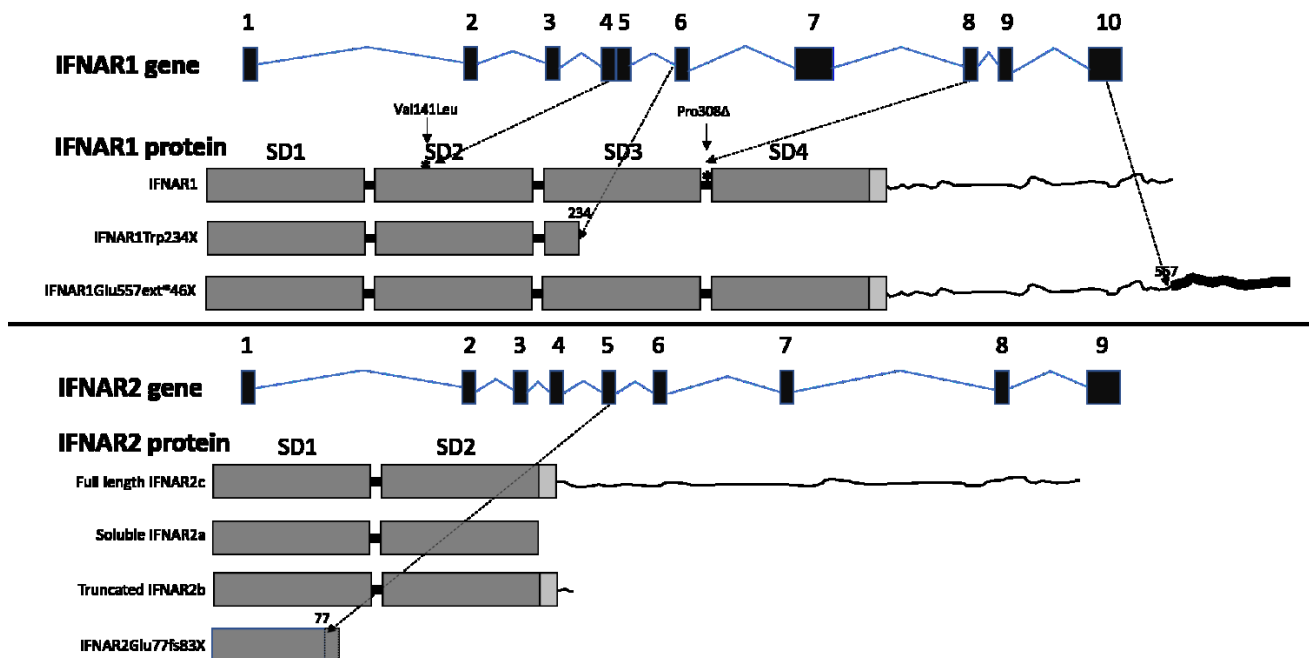


Fig 1. SNPs in IFNAR1 and IFNAR2 with clinical associations. (A) Nonsynonymous SNPs identified in IFNAR1 (blue) and IFNAR2 (orange) and their clinical associations.

Indicated on IFNAR1 and IFNAR2 are the approximate locations of SNPs discussed: A, SNP

rs2257167, IFNAR1Val141Leu; **B**, SNP inducing IFNAR1Val225fs228X and IFNAR1Trp234X; **C**, SNP rs72552343, IFNAR1Pro308Δ; **D**, ENU SNP *macro-1*, IFNAR1Thr315Pro; **E**, SNP inducing IFNAR1Glu557ext*46X; **F**, SNP rs2229201, IFNAR2Phe8Ser and SNP rs1051393, IFNAR2Phe10Val; **G**, SNP rs775739391, IFNAR2Glu77fs83X. Clinical associations include: MS, Multiple Sclerosis; HIV/AIDS, Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome; HBV, Hepatitis B Virus; YF, Yellow Fever; HCV, Hepatitis C Virus; MMR, Measles Mumps Rubella.

Approximate locations of mutated residues are shown on the receptors in red, labelled A-G.

(B) Diagrammatic representation of the genes encoding IFNAR1 (top section) and IFNAR2 (bottom section) showing (top row) approximate exon (boxes)-intron (lines) boundaries of the genes, (second row) the domain structure of the normal encoded proteins, the exon location of SNPs discussed throughout this review (arrows) and a comparison of the mutated receptor structure compared to the normal receptor (comparisons of rows 2, 3 and 4).

Asterisks denote the approximate locations of nonsynonymous SNPs in IFNAR1 that produce proteins of similar length to the normal IFNAR1 receptor. Subdomains (SD) of receptors are shown as black rectangles and identified as SD1-4. The transmembrane domains are shown as grey rectangles. Intracellular domains are shown as unstructured lines. Information on exon-intron boundaries and exon numbers was gained from

<http://asia.ensembl.org/index.html>.

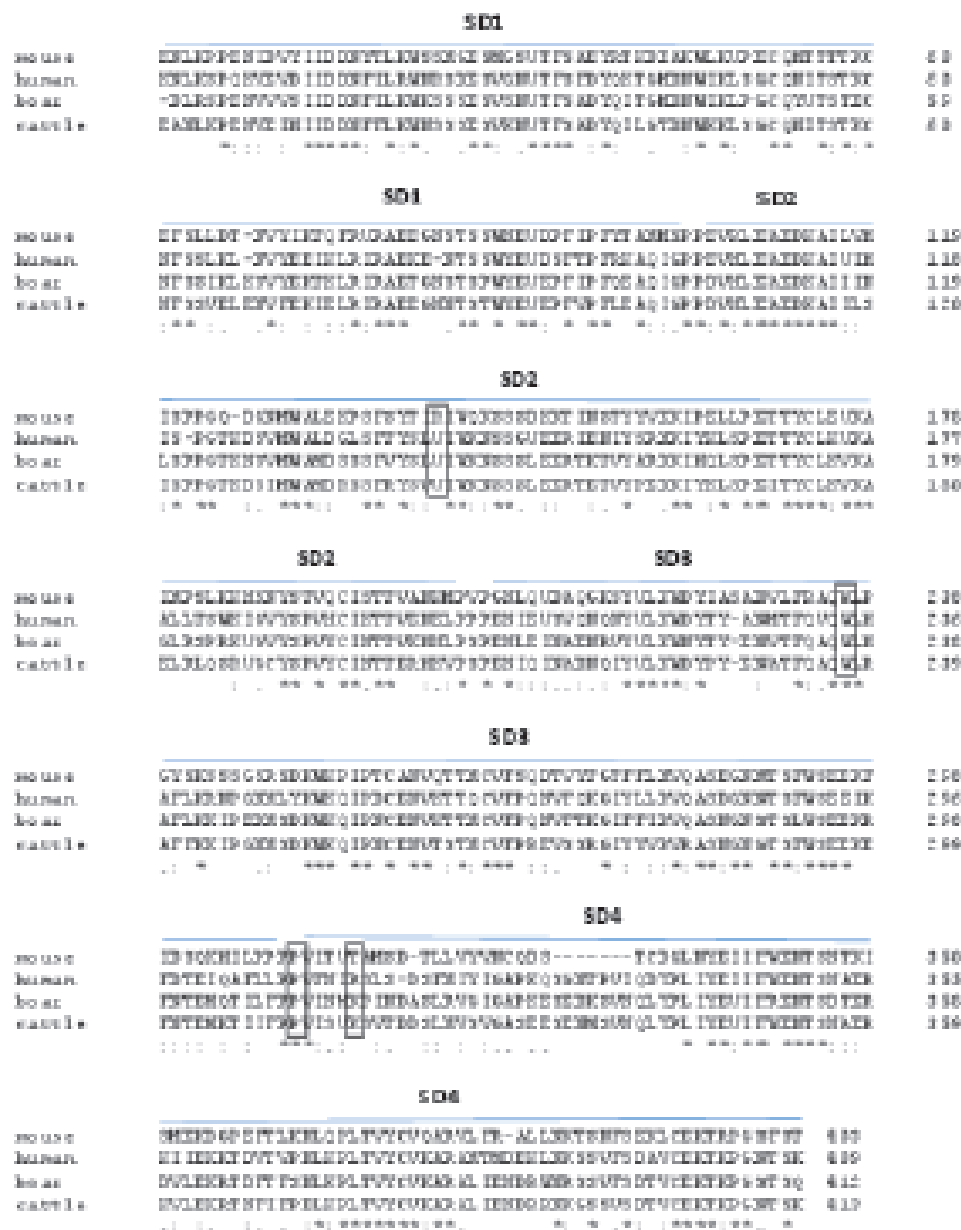


Fig. 2. Locations of SNPs on IFNAR sequences. (A) genome map of in exon-intron boundaries and the translated normal and mutated proteins for IFNAR1 and IFNAR2. (B) Alignment of sequences of mouse, human, boar and cattle IFNAR1-ECD (generated using Clustal Omega). Subdomains (SD) are indicated. Bold boxes indicate the location of disease-associated non-synonymous SNP mutations (see text). GenBank accession numbers: mouse AAH43935.1; human NP_000620.2; boar NP_998937.1; cattle NP_776977.1. (C) Alignment

of IFNAR2-ECDs (generated using Clustal Omega) from mouse and human. Subdomains (SD) are indicated. Bold boxes indicate the location of disease-associated non-synonymous SNP mutations (see text). GenBank accession numbers: mouse AAC53351.1; human AAH02793.1; boar NP_001191704.2; sheep NP_001009342.1.

Figure 3A

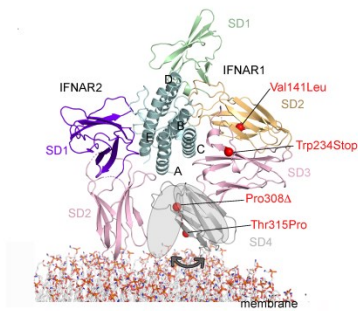


Figure 3C

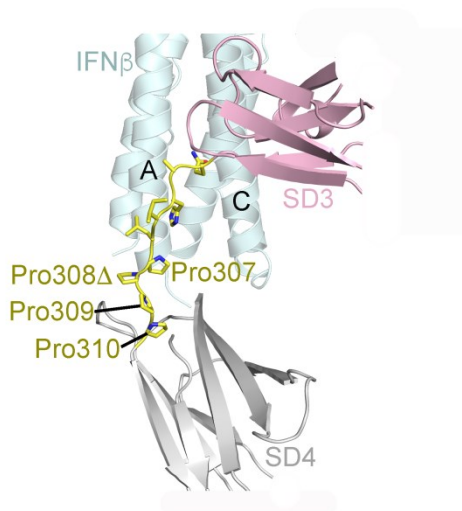
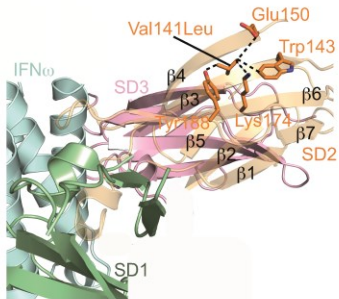


Figure 3B



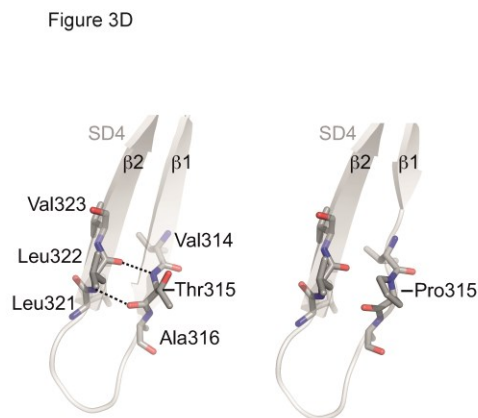
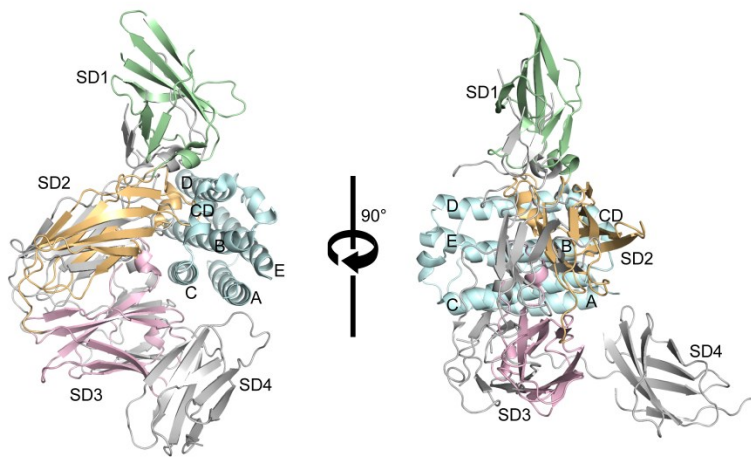


Fig. 3. Model of the functional IFNAR1/IFNAR2/IFN complex on the cell surface

showing locations of SNPs on the receptors. (A) The complex was modelled as a hybrid of the human IFNAR1-SD1-3/IFNAR2/IFN ω with the murine IFNAR1-SD4 (coloured by subdomain (SD)). Murine IFNAR1-SD4 is coloured grey and is rotated by $\sim 16^\circ$ relative to the human IFNAR1, shown by the grey ovals superimposed on SD4. The orthogonal displacement of the IFNAR1-SD4 from SD1-3 aligns this domain with IFNAR2-SD2 and increases the proximity of their transmembrane domains (membrane is depicted as sticks). The highly flexible linker between IFNAR1-SD3 and SD4 and the paucity of contacts between SD4 and IFN enables a high degree of movement for SD4 that we propose is necessary for functional signalling (the arrow indicates a hypothetical movement of IFNAR1-SD4 upon ternary complex formation). The labels for SDs of IFNAR1 (SD1-4) and IFNAR2 (SD1-2) are colour coded as per the SD on these receptors. The helical IFN is positioned

between IFNAR2 (left of the IFN) and IFNAR1 (right of the IFN) is shown in cyan with helices labelled as A-E. The approximate locations of SNPs on IFNAR1 and IFNAR2 are indicated by red spheres and labelled according to the nomenclature given in the text – on IFNAR1 are Val141Leu (B), Pro Δ 308 (C), Thr315Pro (D).

Figure 4A



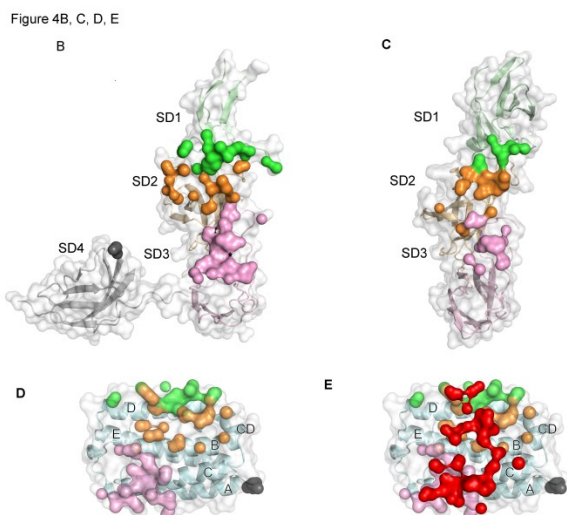


Fig. 4 Overall structure and footprints of IFNAR1 bound to IFN. (A) Two orthogonal views of the complexes between IFNAR1 and IFN are shown. Human IFNAR1 is coloured by subdomain (SD1=green, SD2=orange, SD3=pink). Murine IFNAR1 (overlaid on human IFNAR1) is coloured grey and rotated by $\sim 16^\circ$ relative to the human IFNAR1. Differences between the human and murine receptors are most noticeable on IFNAR1SD2-4. The orthogonal displacement of IFNAR1-SD4 from IFNAR1SD1-3 is most noticeable in the view on the right. (B) Contact atoms of IFN β on murine IFNAR1 mapped to the surface of the receptor. Atoms are coloured according to the subdomain of IFNAR1 to which they bind (SD1=green, SD2=orange, SD3=pink, SD4=grey). (C) Contact atoms of IFN ω on human IFNAR1 mapped to the surface of the receptor. Atoms are coloured by subdomain of IFNAR1 to which they bind (SD1=green, SD2=orange, SD3=pink). (D) Contacted atoms mapped to the surface of murine IFN β (coloured by IFNAR1 subdomain as in (B)). (E) Contacted atoms mapped to the surface of human IFN ω (red) overlaid on the footprint of

murine IFNAR1 on IFN β (coloured by IFNAR1 subdomain as in (B)). The comparison of footprints of IFNAR1 on IFN ω and IFN β shown in (E) highlights regions of overlap or “anchor points” for SD1-3 as well as regions where recognition diverges.

Figure 5A, B

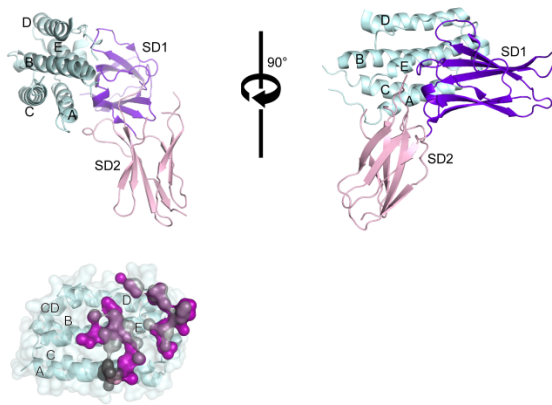


Fig 5. Structural overview of IFNAR2 in complex with IFN. (A) Two orthogonal views of the complexes between IFNAR2 and IFN. Human IFNAR2 is coloured by subdomain (SD1=purple, SD2=pink). (B) Footprint of IFNAR2 on IFN α and IFN ω . The footprint on IFN α is coloured by IFNAR2 subdomain as in (A). (C) The footprint on IFN ω is coloured by IFNAR2 subdomain (SD1=light grey, SD2=dark grey). The overlay of the footprints highlights “hotspots” of conservation on the AB loop and E helix as well points of divergence on the A and E helices. Notably, SD1 dominates the binding interface with SD2 contributing very little surface area.

Table 1: Disease-associated SNPs that result in substitution or deletion of amino acids on the human IFNARs; these SNPs are associated with altered disease susceptibility or resistance.

Recepto	SNP ID	AA change ¹	Condition ²	Phenotype ³	Ref
IFNAR1	rs2257167	Val141Leu	Multiple sclerosis	Increased risk	(43)
			Cerebral malaria	Increased risk	(40)
			HIV/AIDS	Increased risk of progression	(42)
			HBV	Increased risk chronic infection	(37)
			HBV & HCV	HBV-ACLF risk	(36)
			Lung Cancer	Increased risk/pain	(45, 46)
			Female Vitiligo	Increased risk	(44)
Unassigned	Val225fs228 X	Measles/YEL- AVD	Adverse reaction to vaccination	(49)	
Unassigned	Trp234X	Yellow Fever	Adverse reaction to vaccination	(49, 50)	
rs72552343	Pro308Δ	HBV	Increased susceptibility	(50, 127)	
		Tuberculosis	Reduced risk	(50)	
Unassigned	Glu557ext*46	HLH, CMV	Increased risk	(61)	

		X	/iremia		
IFNAR2	rs1051393	Phe10Val	HCV	Increased risk	(49, 63)
			Persistent HCV	Increased risk	(63, 64)
			Multiple sclerosis	Increased risk	(43, 64)
	rs2229201	Phe8Ser	HCV	More likely to have had an SVR	(43, 62)
	rs775739391	Glu77fs83X	Measles	Adverse reaction to /accination	(68)

¹ All amino acid residues are numbered according to its position in the mature IFNAR protein. fs, frameshift; stop, stop codon; del, deletion; X, premature stop codon; ext*, extension of protein by number of residues indicated.

² HIV/AIDS, Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome; HBV, Hepatitis B Virus; HCV, Hepatitis C virus; YEL-AVD, Yellow fever vaccine-associated viscerotropic disease; MMR, Measles- Mumps-Rubella; HLH, hemophagocytic lymphohistiocytosis; CMV, cytomegalovirus.

³ HBV-ACLF, Acute-on-Chronic Liver Failure in HBV; SVR, Sustained virologic response,

Table 2: Residues identified in the IFNAR-IFN interface by mutational studies and protein crystallography.

Receptor	Ligand	'Hot-spot' residues	Minor ligand binding residues	Ref
IFNAR1	hIFN α 2-YNS	Tyr70, Phe238	Phe96, Leu132, Glu133, Leu134, Ser136, Tyr157, Asn242	(48, 128)
	mIFN β	Tyr70, Tyr98, Tyr240, Tyr274	Asp66, Thr67, Asn68, Phe97, Asn128, Leu132, Glu133, Lys134, Ser182, Leu183, Lys184, Pro238, Gly239, Ser243, Ser244, Ser245, Thr275, Thr277, Asp300, Gln302, Leu335	(21)
	hIFN ω	Tyr70, Phe238	Asn68, Fhe96, Leu131, Asp132, Leu134, Thr181, Ser182, His236, Phe238, Asn242	(48)
IFNAR2	hIFN α 2-YNS	Met46	Thr44, Ile45, Ser47, Glu50, Leu52, His76, Glu77, Val80, Trp100, Ile103	(48)
	hIFN β		His76, Asn98, Trp100	(114)
	hIFN ω		Thr44, Ile45, Met46, Glu50, Val80, Trp100, Ile103	(48)