A proline deletion in IFNAR1 impairs IFN-signaling and underlies increased resistance to tuberculosis in humans

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Type I interferons (IFN), best known for their anti-viral functions, have been shown to impair host resistance to intracellular bacteria in mice. However, the precise role of type I IFN signaling in bacterial infection in humans is unclear. Here, we show that genetic variation in the human IFNAR1 gene is associated with decreased susceptibility to tuberculosis and an increased risk of viral hepatitis in Chinese populations. Receptor mutagenesis and cell signaling studies establish that the IFNAR1 mutation corresponding to a proline deletion in the hinge region of the membrane-proximal domain of IFNAR1 decreases the binding affinity of IFNAR1 to IFN-β, impeding type I IFN signaling. Our findings suggest that IFNAR1 signaling underlies an increased risk of tuberculosis in humans and reveals a function for the IFNAR1 inter-domain region in cytokine–cytokine receptor interaction and signal transduction.
The type I interferon (IFN) family consists of ~20 different members including multiple IFN-α subtypes and IFN-β. The signaling complex utilized by type I IFNs comprise IFN alpha and beta receptor subunit 1 and 2 (IFNAR1 and IFNAR2). Ligand engagement of the extracellular domains of the receptor complex induces the phosphorylation of signal transducer and activator of transcription (STAT) molecules, which subsequently activate a set of Interferon Stimulated Genes (ISG). Structurally, IFNAR1 is unique amongst the class II helical cytokine receptors as its extracellular domain is comprised of a four-domain architecture, referred to as subdomains (SD). Although the minimal ligand-binding region is localized to the membrane-distal portion (SD1-SD3), the membrane-proximal domain (SD4) is required to undergo a conformational change necessary for signal transduction across the cell membrane.

Similar to many class II helical cytokine receptors, IFNAR1 has paired proline residues in hinge regions between individual extracellular domains. Paired proline residues located in such non-helical linker regions are hypothesized to increase local structural rigidity.

Type I IFNs are established as important in anti-viral immunity; however, these cytokines also impair immune responses to various pathogens. Mice deficient in IFNAR1 (Ifnar1−/−) have reduced pathogen loads in response to intracellular bacterial infection, and administration of type I IFN-inducing poly: (IC) exacerbates listeriosis and tuberculosis (TB) in wild-type (WT), but not Ifnar1−/− mice. In contrast to animal studies, evidence that IFNAR1 signaling contributes to TB disease susceptibility in humans is unclear, although several clinical studies have linked the blood interferon signature to detrimental clinical outcome. Here we show that a rare IFNAR1 genotype is associated with decreased risk of TB in Chinese populations and the mutation decreases the magnitude of IFN-β-mediated ISG induction in cells from individuals heterozygous for the SNP. We further demonstrate that the SNP decreases type I IFN signaling by reducing the overall binding affinity of IFN-β for IFNAR1. These findings suggest a host-detrimental function for type I IFN signaling in human TB. As we show that the mutation, which occurs at a non-cytokine binding region of IFNAR1, weakens the cytokine receptor interaction, our findings also suggest a function for the membrane-proximal domain in type I IFN binding and signaling.

Results

ISG induction is associated with active TB in Chinese population. As an initial step in understanding the function of type I IFNs in human TB, we examined the expression of ISGs in the leukocytes of healthy controls (HC), latent TB infection (LTBI) subjects and TB patients. LTBI subjects were positive for Interferon-Gamma Release Assay (IGRA) testing, but with no clinical symptoms of active TB and normal chest radiography. In agreement with previous studies performed in African populations, we observed that PBMCs of active TB patients expressed significantly higher levels of ISGs than LTBI subjects in Chinese populations.

In addition, blood samples were also collected from a group of active pulmonary TB patients before and after the initiation of the standard 2 months of isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E) during intensive phase and 4 months of HR in continuation phase (2HRZE/4HR) treatment regimen recommended by the WHO guideline. We observed that the elevated ISG expression declined rapidly after initiation of anti-TB drug treatment.

SNP rs72552343 in IFNAR1 reduces risk of developing active TB. To understand the function of type I IFN signaling in TB susceptibility in humans, we determined the association of IFNAR1 genetic polymorphisms and the risk of developing active TB disease in Chinese populations. Human IFNAR1 is located on chromosome 21 adjacent to IL10RB and IFNGR2 (Supplementary Fig. 1a). In this study, six single-nucleotide polymorphisms (SNP) (Supplementary Table 1) in the gene encoding IFNAR1 were examined for their association with clinical TB. The donor selection criteria and SNP genotyping using the Sequenom MassARRAY platform have been described in our previous study. Active TB and HC patients were recruited at Shenzhen, a south-eastern city in Guangdong Province. The cohort includes 1533 active TB patients and 1445 healthy controls. Among the 1533 active TB patients, 1432 were diagnosed with pulmonary TB (PTB), 101 with extra-pulmonary TB (ETB). We observed that...
virus surface antigen (HBsAg)-positive hepatitis B (HepB) rs72552343 genotype distribution was determined in hepatitis B pathogenesis in viral infection in humans. To this end, the next examined whether SNP rs72552343 in Since type I IFNs are known to mediate anti-viral immunity, we signiﬁed disease in active TB (multiplicative model: p = 0.0002; dominant model: p = 0.0003) (Table 1). Further sequencing analysis conﬁrmed that SNP rs72552343 is associated with the in-frame deletion of nucleotides TCC (Supplementary Fig. 1b). To validate this genetic association, the distribution of the SNP rs72552343 genotype was further determined in a second TB cohort. We found that while the rs72552343 TCC deletion was associated signiﬁcantly with decreased risk of both pulmonary (PTB vs. HC, OR: 0.47; p < 0.0001; dominant model) and extra-pulmonary TB disease (ETB vs. HC, OR: 0.40; p = 0.005; dominant model), it did not preferentially influence the development of the either type of the disease (Supplementary Table 3). Similarly, the host protective effect of TCC deletion on pulmonary TB was present in TB patients who were mycobacterial culture and sputum smear positive or negative (Supplementary Tables 4 and 5). In addition, analysis of high-resolution computed tomography (HRCT) ﬁndings revealed that pulmonary TB patients carrying rs72552343 TCC/Del exhibited reduced risk of developing pulmonary cavities compared to their TCC/TCC counterparts (cavity− vs. cavity+, p = 0.02; multiplicative and dominant models, Supplementary Table 6).

To investigate further the association between SNP rs72552343 and pulmonary pathology in TB patients, HRCT images of pulmonary TB patients enrolled in Shenzhen study were further assessed and scored based on the patterns, proﬁsion and distribution of pulmonary abnormalities including cavity formation.25 This analysis revealed that pulmonary TB patients carrying TCC/Del genotype showed signiﬁcantly lower HRCT scores (less severe TB disease) than those with the common allele (Fig. 2a). When radiographic data collected before and 2 years after treatment were compared in a subset of the TB patients, we found that IFNAR1 TCC deletion was also associated with a more favorable disease outcome following a standard 2HRZE/4HR treatment regimen (Fig. 2b). Altogether, these data establish an association between type I IFN receptor signaling and increased tissue pathology in active pulmonary TB.

Table 1 Association of IFNAR1 rs72552343 and tuberculosis susceptibility

| Cohort       | Genotype | HC | TB       | Multiplicative | Dominant |
|--------------|----------|----|----------|---------------|---------|
|              |          |    |          | p<sup>b</sup> | OR (95% CI)<sup>c</sup> |          | p      | OR (95% CI) |
| Discovery    |          |    |          |               |         |          |         |
| TCC/TCC     | 1378 (95.4) | 1499 (97.8) | 0.0002 | 0.46 (0.31-0.70) | 0.0003 | 0.47 (0.30-0.71) |
| TCC/DEL     | 66 (4.6) | 34 (2.2) |               |         |         |         |
| DEL/DEL     | 1 (0.0) | 0 (0.0) |               |         |         |         |
| Validation  |          |    |          |               |         |          |         |
| TCC/TCC     | 1043 (96.2) | 819 (98.4) | 0.004 | 0.41 (0.21-0.76) | 0.004 | 0.40 (0.21-0.75) |
| TCC/DEL     | 41 (3.8) | 13 (1.6) |               |         |         |         |
| DEL/DEL     | 0 (0.0) | 0 (0.0) |               |         |         |         |
| Combined    |          |    |          |               |         |          |         |
| TCC/TCC     | 2421 (95.7) | 2318 (98.0) | <0.0001 | 0.45 (0.32-0.64) | <0.0001 | 0.45 (0.32-0.64) |
| TCC/DEL     | 107 (4.3) | 47 (2.0) |               |         |         |         |
| DEL/DEL     | 1 (0.0) | 0 (0.0) |               |         |         |         |

*a* Number of samples with genotype frequency shown in parentheses

*b* Significant p values (<0.05) are shown in bold

*c* OR odds ratio, numbers in parentheses following OR are 95% confidence intervals

SNP rs72552343 in IFNAR1 increases hepatitis B susceptibility. Since type I IFNs are known to mediate anti-viral immunity, we next examined whether SNP rs72552343 influenced disease pathogenesis in viral infection in humans. To this end, the rs72552343 genotype distribution was determined in hepatitis B virus surface antigen (HBsAg)-positive hepatitis B (HepB) patients and HC recruited for a previously published study.24. In contrast to mycobacterial infection, IFNAR1 rs72552343 TCC/ Del genotype was observed to be associated with signiﬁcantly increased susceptibility to viral hepatitis (multiplicative model: p = 0.01; dominant model: p = 0.02, Table 2). Therefore, IFNAR1-signaling can differentially regulate resistance to intracellular bacterial and viral infection in humans.

Table 2 Association of IFNAR1 rs72552343 and viral hepatitis susceptibility

| Genotype | HC | HBV | Multiplicative | Dominant |
|----------|----|-----|---------------|---------|
|          |    |     | p<sup>b</sup> | OR (95% CI)<sup>c</sup> |         | p      | OR (95% CI) |
| TCC/TCC  | 857 (95.9) | 791 (93.3) | 0.01 | 1.70 (1.12-2.58) | 0.02 | 1.66 (1.09-2.55) |
| TCC/DEL  | 37 (4.1) | 55 (6.5) |               |         |         |         |
| DEL/DEL  | 0 (0.0) | 2 (0.2) |               |         |         |         |

*a* Number of samples with genotype frequency shown in parentheses

*b* Significant p values (<0.05) are shown in bold

*c* OR odds ratio, numbers in parentheses following OR are 95% confidence intervals

TCC deletion in IFNAR1 is associated with reduced TB pathology. We next analyzed the association of rs72552343 with the clinical manifestations of TB diseases, sputum smear and mycobacterial culture positivity and lung cavity formation in the combined TB cohort. We found that rs72552343 TCC deletion was associated signiﬁcantly with decreased risk of both pulmonary (PTB vs. HC, OR: 0.47; p < 0.0001; dominant model) and extra-pulmonary TB disease (ETB vs. HC, OR: 0.40; p = 0.005; dominant model), it did not preferentially influence the development of the either type of the disease (Supplementary Table 3). Similarly, the host protective effect of TCC deletion on pulmonary TB was present in TB patients who were mycobacterial culture and sputum smear positive or negative (Supplementary Tables 4 and 5).
TCC deletion in IFNAR1 impairs type I IFN signaling in PBMCs. To determine whether IFNAR1 SNP rs72552343 plays a role in the cellular response to IFN stimulation, PBMCs from individuals carrying WT (TCC/TCC) or mutant (TCC/Del) IFNAR1 genotypes were stimulated with recombinant human IFN-β, IFN-α, or both cytokines, and ISG expression analyzed using qRT-PCR. We observed that MX1, IFT1, OAS1, STAT2, and STAT1, which were highly sensitive to IFN-β induction, were up-regulated to a lesser extent in cells carrying the TCC deletion than in their WT counterparts following IFN-β stimulation (Fig. 3). In contrast, the upregulation of CXCL10 and IRF1 driven predominately by IFN-γ at this time-point was not affected by the IFNAR1 mutation. In addition, there was no significant difference in the level of IFNAR1 or IFNAR2 between genotypes. These findings collectively suggested TCC deletion is associated with defective type I, but not type II, IFN signaling.

As IFNAR1 signaling can regulate type I IFN production26,27, we examined whether the IFNAR1 mutation has an impact on the production of type I IFNs. We first compared the levels of IFN-α and IFN-β in the plasma of HC and TB patients carrying TCC/TCC or TCC/Del genotypes. Although IFN-α was not measurable in plasma, a low level of circulating IFN-β was detected in all TB cases irrespective of their genotype (Supplementary Fig. 2a). Similarly, a comparable induction of IFN-α and IFN-β was observed in the culture supernatants of M. tuberculosis-infected macrophages carrying TCC/TCC or TCC/Del IFNAR1 (Supplementary Fig. 2b), suggesting that the TCC/Del in IFNAR1 does not appear to impact type I IFN production in TB patients and M. tuberculosis-infected macrophages.

TCC deletion in IFNAR1 decreases type I IFN signal transduction. To establish definitively that the identified IFNAR1 deletion affects type I IFN signaling, mammalian expression vectors encoding human WT IFNAR1 and IFNAR1Del were generated for transfection studies. Together with an IFNAR2 encoding vector and an ISRE-driven luciferase reporter plasmid, the WT or mutant IFNAR1 vectors were transfected into Ifnar1−/− mouse embryonic fibroblasts (MEFs). Ifnar1−/− MEFs were used to ensure that the activation of the IFN signaling pathway by human IFNs depends exclusively on the transfected IFNAR1. Because both IFN-β and IFN-α can signal via IFNAR11, we also included IFN-α in this series of experiments to determine whether the signaling defect resulting from the TCC deletion is restricted to IFN-β stimulation. Upon stimulation with human IFN-α or IFN-β, WT IFNAR1-transfected cells exhibited significantly stronger luciferase activity compared to their IFNAR1Del-transfected counterparts, whereas IFN-γ induced minimal luciferase activity, similar to levels seen in the untransfected cells (Fig. 4a). Similarly, WT IFNAR1-transfected MEFs expressed significantly higher levels of IFN-inducible Isg15, Ift11, and Oasl than the IFNAR1Del-transfected cells when measured at 3 and 6 h after IFN stimulation (Fig. 4b). The reduced IFN-inducible response in IFNAR1Del-transfected MEFs correlated with similarly impaired STAT1 phosphorylation following IFN-β stimulation (Fig. 4c and d).

To confirm the above findings in a human system, we knocked down endogenous IFNAR1 in HEK293 cells using CRISPR/Cas9 gene-editing technology and then transfected the cells with human WT IFNAR1 or IFNAR1Del plasmids together with an ISRE-driven luciferase reporter plasmid. Similar to the studies
performed using MEFs, following type I IFN stimulation, HEK293 cells transfected with WT IFNAR1 exhibited significantly stronger luciferase activity compared to IFNAR1Del-transfected counterparts (Fig. 5a). WT IFNAR1-transfected cells also expressed significantly higher levels of IFN-inducible ISG15, IFIT1, and OAS1 compared to the IFNAR1Del-transfected counterparts when measured at 6 h after IFN stimulation (Fig. 5b). Altogether, these biochemical studies performed using both murine and human cells provide direct evidence demonstrating that the TCC deletion and the resulting deletion of proline 335 (Pro335) impairs IFNAR1-dependent type I IFN signaling.

**IFNAR1** TCC deletion decreases binding affinity of IFN-β to IFNAR1. The TCC deletion in IFNAR1 results in the subsequent deletion of one of the paired proline residues (Pro335) located in the inter-domain hinge between SD3 and SD4 of human IFNAR1 (Fig. 6a). Evidence from the literature suggests that deletion of Pro335 from IFNAR1 would increase flexibility of the hinge domain between SD3 and SD4 of IFNAR1, thereby altering ligand-binding kinetics. To test this hypothesis, we generated recombinant forms of the hIFNAR1-extracellular domain (ECD) and also a form with the Pro335 deletion (IFNAR1-ECD-Del) in an insect cell expression system, and used these to assess the effect of Pro335 deletion on hIFN-β affinity for IFNAR1 using surface plasmon resonance (SPR). We observed in three independent experiments that deletion of Pro335 from IFNAR1 significantly reduced the overall affinity of the ligand–receptor interaction (WT IFNAR1 affinity was determined to be 15.8 ± 3.4 nM (mean ± standard deviation, n = 3 independent experiments) compared to IFNAR1Del affinity at 28.1 ± 3.1 nM), whereas the rate of association and dissociation of hIFN-β to and from the receptor were not significantly different for both WT IFNAR1 and IFNAR1Del (Fig. 6b, c).

**Discussion**

Animal studies have suggested that type I IFNs can impair resistance to a diverse range of pathogens. In particular, immunity against intracellular bacteria is often compromised by type I IFNs. However, whether type I IFN-dependent regulatory mechanisms dictate the outcomes of bacterial infection in humans are unknown. In this study, we have combined genetic and biochemical approaches to elucidate type I IFN function in human TB. We identify that a genetic variant in the human IFNAR1 gene (the deletion of nucleotides TCC) decreases type I IFN signaling and reduces the risk of active TB. Moreover, pulmonary TB patients carrying the TCC/Del genotype exhibit less severe tissue pathology in their lungs than their TCC/TCC counterparts. Finally, the same IFNAR1 mutation is associated with increased susceptibility to viral hepatitis, revealing a
pathway leads to distinct disease outcomes in humans. As IFNs are pleiotropic cytokines, the downstream outcome of type I IFN signaling will depend on the type and location of the cells activated by the cytokines. It is also possible that the major function of type I IFNs in infection in vivo is directed at the host rather than the pathogen. Interestingly, our data suggest that type I IFN signaling is associated with more severe immunopathology in pulmonary TB. Discovery of additional genetic associations of IFNAR1 signaling components and susceptibility of TB and other infectious diseases in future studies will assist in validating the observations reported here and in dissecting the complex function of IFNs in humans. It is also worth noting that not all identified IFNAR1 SNPs associated with viral susceptibility were associated with increased resistance to mycobacterial infection. IFNAR1 SNP rs2843710, shown previously to exhibit increased susceptibility to enterovirus infection, did not display a significant association with active TB in the current study (Supplementary Table 1).

The extracellular domains of class II helical cytokine receptors, of which IFNAR1 is a member, are composed of discrete subdomains separated by distinctive poly-proline, inter-domain hinges. The presence of such poly-proline hinges between functional receptor subdomains leads to structural rigidity of the protein thereby dictating the conformational state of the receptor. Factors that perturb protein rigidity, including mutation or substitution of proline residues in inter-domain hinge regions can increase protein flexibility thereby decreasing ligand-binding affinity and altering protein conformational states and protein function. Although the minimal ligand-binding domain of IFNAR1 is limited to its three membrane-distal subdomains (SD 1–3), the membrane-proximal subdomain (SD4) of IFNAR1 is required to undergo a conformational change for optimal IFN-induced signal transduction across the cell membrane. Given this evidence from the literature, we hypothesized that deletion of Pro335 from IFNAR1-EC-Del would reduce the rigidity of the inter-domain hinge between IFNAR1 SD3 and SD4, thereby increasing inter-domain flexibility and altering the IFN-β binding kinetics. The fact that IFNAR1-EC-Del, compared to its WT counterpart, shows reduced affinity for IFN-β strongly suggests that deletion of Pro335 from IFNAR1-EC-Del has indeed increased flexibility of the receptor. Taken together, our results suggest that SNP rs72552343 has reduced IFNAR1 functionality by altering IFN binding kinetics and thereby modifying receptor response upon ligand engagement, reducing the magnitude of STAT1 activation and subsequent ISG induction measurable from the mutated receptor.

It remains to be determined how the proline deletion attenuates IFNAR signaling in the heterozygous individuals. There is, however, a precedent in the literature for the heterozygous expression of a SNP that results in a missense mutation in human IFNAR1 to alter the function of the receptor. SNP rs2257167, which causes substitution of Leucine 168 to Valine in IFNAR1, has been the focus of a number of studies. For example, in a severe malaria susceptibility study, the presence of SNP rs2257167 in the heterozygous state was associated with increased susceptibility to this disease. There are other examples where the heterozygous state of SNPs in cell surface receptors, such as in IFNGR1, a helical cytokine receptor related to IFNAR1, is also associated with disease outcome.

As only virulent mycobacteria induce type I IFN production in infected mice and human macrophages, it is hypothesized that type I IFN signaling is associated with mycobacterial virulence and increased host susceptibility. Although the exact mechanisms by which type I IFNs exacerbate TB are not fully understood, IFNAR1 deficiency or therapeutic inhibition of type I IFN production have been shown to be host beneficial in
resistance to *M. tuberculosis* infection in mice. Our findings here demonstrate that reduced type I IFN signaling resulting from a naturally occurring mutation in IFNAR1 decreases the risk of developing active TB in humans. Together, these experimental and clinical studies highlight a pivotal role for type I IFN signaling in determining the outcome of mycobacterial infection. Identification of the molecular mechanisms mediating the anti-viral and pro-bacterial functions of type I IFNs may lead to a better understanding of basic IFN biology, pathogenesis of human infectious diseases, and assist in developing novel therapeutics.

**Methods**

**Study populations.** We sampled three from case-control cohorts. All subjects were genetically unrelated Southern Han Chinese adults. Active TB was defined based on the WHO guideline for the diagnosis of non-HIV related TB with the following criteria: clinical signs and symptoms, chest radiography, acid-fast bacilli (AFB) identification (spumtn smear or *M. tuberculosis* culture positive), and response to anti-TB chemotherapy. Patients with allergic diseases, diabetes, cancer, immune-compromised conditions, and HIV infection were excluded from the study.

The discovery TB cohort includes 1533 active TB patients and 1445 HC recruited at Shenzhen Third People’s Hospital, Shenzhen, Guangdong Province. Among the 1533 active TB patients, 1432 were diagnosed with pulmonary TB, 101 with extra-pulmonary TB including tuberculous lymphadenitis (*n* = 62), tuberculosis meningitis (*n* = 13), and osteoarticular TB (*n* = 26). The healthy controls were asymptomatic individuals with negative T cell reactivity to *M. tuberculosis*-specific antigens tested in an in-house Interferon-Gamma Release Assay (iGRA) and normal chest radiography. For the validation study TB cohort, 832 active TB cases and 465 extra-pulmonary TB and 1084 controls were recruited at West China Hospital, Sichuan University in Chengdu, Sichuan province. Individuals with Tibetan background have been excluded from the study.

Hepatitis B (HepB) study includes 848 patients and 894 controls. HepB cases were diagnosed based on the Guideline of the Prevention and Treatment of Chronic Hepatitis B published by the Ministry of Health of China. All HepB patients were tested positively for the HBsAg in the serum (Zhuhai Livzon Diagnostics, China) and displayed elevated Alanine transaminase levels (ALT > 40 IU/L). The HC were individuals without clinical history of TB, HepB, HepC and HIV infection. All HC displayed normal ALT levels and were tested negatively for serum HBsAg, HepC and HIV antibodies to HBV (Advia, USA) and HIV (Abbott, USA) and normal chest radiography (Zhuhai Livzon Diagnostics, China). All individuals were recruited at Shenzhen Third People’s Hospital, Shenzhen, Guangdong Province. The characteristics of the TB and HepB study populations are shown in Supplemental Table 2.

**Ethics Statement.** Blood samples were collected and analyzed after written informed consent was obtained from participants and with approval of Shenzhen Third People’s Hospital Ethics Committee (Reference No. 2012-006). The study was approved by the Ethics Committee of West China Hospital, Sichuan University (Reference No. 198 (2014)).

**SNP selection and genotyping.** Genomic DNA was prepared from PBMC using QIAamp DNA Blood MiniKit (Qiagen, Hilden, Germany). To select SNP candidates in IFNAR1 gene, we used a Position Weight Matrices (PWM) SCAN algorithm to scan IFNAR1 sequence in Jaspar, UniPROBE, TRANSFAC and PITA databases. For the SNP within the putative binding sites, *v* value was calculated to reflect the change of binding scores between the putative binding site and putative binding protein, and then converted into a *p*-value based on the FastPval program, the SNP with *p*-value < 0.01 was selected for genotyping, in addition, non-synonymous substitutions in the exon region were also selected. In TB discovery study, three SNPs in the promoter (*rs2348191* T>G, *rs2348370* C>G, *rs17875752* G>T), 1 SNP in exon (*rs7255343* TCC>DEL) and 2 SNPs in the intron (*rs1012334* A>T, *rs1041868* G>A) of IFNAR1 (Supplementary Table 1) were genotyped in healthy controls and TB patients using the MassARRAY system (Sequenom, San Diego, CA). The relative height (intensity) of the peaks and the signal-to-noise (SNR) ratio were analyzed using Caller software to call genotypes in real-time. After cluster analysis using Typer software, manual curation of spectra genotyped in health controls and TB patients using the MassARRAY system (Sequenom). The cell lines were cultured in 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 μM glutamate, and 10 mM HEPEs. The recombinant human IFN-α/2a, IFN-β and IFN-γ were purchased from PBL Assay Science.

**Cell culture and in vitro stimulation of PBMCs.** PBMCs were isolated by density gradient centrifugation over Ficoll-Hypaque. Plural fluid mononuclear cells were obtained and cryopreserved. FC scanning were obtained on 50–100 μl of thawed PBMCs by 200 ml of pleural fluid from TB patients with pleural effusions at 300×g for 5 min. For ISG expression analysis, PBMCs isolated from the individuals carrying rs7255343 TCC/TC or TCC/DEL genotypes were cultured in a 96-well plate (2×10^5^ cells/well with medium only, human IFN-β (100 IU/ml), human IFN-γ (100 IU/ml) or both cytokines for 6 and total RNA isolated for gene expression analysis using qRT-PCR.

**Human macrophage culture and *M. tuberculosis* infection.** PBMCs were obtained from healthy donors with either TCC or TCC/DEL genotype. Monocytes positively selected from PBMCs using anti-CD14 magnetic beads (Miltenyi Biotec) were cultured in complete RPMI 1640 medium for 7 days. Recombinant human M-CSF (10 ng/ml, PeproTech) was added on days 0, 2, and 4. For infection with mycobacteria, differentiated macrophages seeded in 24-well plates (2×10^5^ cells per well in 0.5 ml antibiotic-free medium) were exposed to *M. tuberculosis* H37Rv at a multiplicity of infection of 10. After 6 h of incubation at 37 °C, the cells were washed three times with warm antibiotic-free medium to remove extracellular bacteria. The supernatants of macrophage cultures were collected 24 h post-infection and the levels of type I IFNs determined using high sensitivity human IFN-β and IFN-α subtype ELISA kits (PBL Assay Science).

**Transfection in MEFs.** *Ifnar1*+/− MEFs, seeded in 24-well plates (4×10^5^ cells/well) 12 h earlier, were co-transfected with plasmid DNA pEFS-BOS-hIFNAR1-WT (0.1 μg) or pEFS-BOS-hIFNAR1-DEL (0.1 μg) vector, together with pEFS-BOS-hIFNAR2 (0.1 μg), ISRE reporter (0.2 μg), and pRL-TK control vector (0.1 μg) using Lipofectamine 2000 reagent (Life Technology). Twenty-four hours after transfection, the cells were cultured with 20 μl human IFN-α, IFN-γ, or IFN-τ. In some experiments, the transfected cells were lysed in 100 μl passive lysis buffer (Promega) after 18 h IFN stimulation and the lysates assayed for both the firefly and Renilla luciferase activities using the dual-luciferase reporter assay system (Promega). Firefly luciferase was normalized against Renilla luciferase activity (F/R ratio) to account for variation in cell numbers and viability. Alternatively, transfected and IFN stimulated *Ifnar1*−/− MEFs were lysed at indicated time points to extract total RNA and protein for analyzing ISG expression by qRT-PCR and STAT1 phosphorylation by Western blot, respectively.

**CRISPR/Cas9 gene editing and transfection.** To determine the role of IFNAR1, we used CRISPR/Cas9 gene editing technology. To ensure CRISPR/Cas9 specifically edited endogenous *Ifnar1* gene, but not open reading frame-containing, transfected exogenous *Ifnar1* constructs, we designed a gRNA (5′-GTGGAGGAGTCTACGTCAGGTC-3′) using CRISPR Design Tool developed by the laboratory of Feng Zhang, MIT (http://crispr.mit.edu/) to target intron 8 of *hIfnar1*. The designed sequence of crp0691 (PCL-IFNAR1 intron 8) was inserted into the plasmid pRRL-U6-sgRNA (qRT-PCR) or HIFNAR1 intron 8. HEK 293T cells were transfected with Cas9-expressing vector pRLR-Cas9-HA-NLS and pRRL-U6-sgRNA-8 using Lipofectamine 2000 reagent. Some of the endogenous *Ifnar1*-silenced cells were co-transfected with pEFS-BOS-hIFNAR1-WT or pEFS-BOS-hIFNAR1-DEL vector, with ISRE reporter and pRL-TK control vectors. After 48 h, the cells were cultured with 100 μg/ml human IFN-α, IFN-β, or IFN-γ and luciferase activities determined at 18 h post-transfection as described above.
Insect cell culture
52. All purified clones of hIFNAR1-ECD and hIFNAR1-ECD-Del in modified baculovirus expression systems (Bac-to-Bac Expression System manual, Life Technologies). Recombinant forms of the extracellular domain (ECD) of hIFNAR1 was amplified from a clone of full-length hIFNAR1 (GenBank accession number CAA42992.1). Amplification of the hIFNAR1-ECD was directed by specific forward (5′-GCCGGAGTCAAAATCTAATACTCCTCAAA-3′) and reverse (5′-GGGAGGATCCTAGATGATGGATATGGTCG-3′) primers. After amplification and digestion of the PCR product with BamHI (5′-end) and EcoRI (3′-end), the amplified fragment was cloned into a modified pFastBac1 vector sequence, and then used in the Bac-to-Bac Expression System (Life Technologies) to obtain recombinant proteins with an N-terminal 6xHis tag as described.

Data were calculated by the 2−ΔΔCT method using 18S or GAPDH as the housekeeping genes. For statistical analysis, one-way analysis of variance (ANOVA) with Newman–Keuls multiple comparison test was used to compare the differences among multiple groups. Student’s t-test was used to compare the differences between two experimental groups. All statistical tests were performed using Prism 6.0 (GraphPad). p < 0.05 is considered statistically significant.

Data availability
The data supporting the findings of this study are available within the article and its Supplementary Information files, or are available from the authors upon request.

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References
1. de Weerd, N. A., Samarajiwa, S. A. & Hertzog, P. J. Type I interferon receptors: biochemistry and biological functions. J. Biol. Chem. 282, 20053–20057 (2007).
2. O’Connor, R. M. et al. Type 1 interferon production enhances susceptibility to Listeria monocytogenes infection. J. Exp. Med. 200, 535–540 (2004).
3. McNab, F., Mayer-Barber, K., Sher, A., Wack, A. & O’Garra, A. Type 1 interferons in infectious disease. Nat. Rev. Immunol. 15, 87–103 (2015).
4. Ng, C. T., Mendoza, J. L., Garcia, C. K. & Oldstone, M. B. Alpha and beta type 1 interferon signaling: passage for diverse biologic outcomes. Cell 164, 349–352 (2016).
5. Auerbuch, V., Brockstedt, D. G., Meyer-Morse, N., O’Riordan, M. & Portnoy, D. A. Mice lacking the type I interferon receptor are resistant to Listeria monocytogenes. J. Exp. Med. 200, 527–533 (2004).
6. Carrero, J. A., Calderon, B. & Unanue, E. R. Type I interferon sensitizes lymphocytes to apoptosis and reduces resistance to Listeria infection. J. Exp. Med. 200, 535–540 (2004).
7. Teles, R. M. et al. Type 1 interferon production enhances susceptibility to Listeria monocytogenes infection. J. Exp. Med. 200, 437–445 (2004).
8. Ordway, D. et al. Hypervirulent Mycobacterium tuberculosis strain HN878 induces a potent T1H1 response followed by rapid downregulation. J. Immunol. 179, 522–531 (2007).
9. Stifter, S. A. & Feng, C. G. Interfering with immunity: detrimental role of type I IFNs during infection. J. Immunol. 194, 2455–2465 (2015).
10. Dorhoi, A. et al. Type I IFN signaling triggers immunopathology in tuberculosis-susceptible mice by modulating lung phagocyte dynamics. Eur. J. Immunol. 44, 2380–2393 (2013).
11. Antonelli, L. R. V. et al. Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. J. Clin. Invest. 120, 1674–1682 (2010).
12. Berry, M. P. et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature Genet. 46, 973–977 (2014).
13. Teles, R. M. et al. Type 1 interferon suppresses type II interferon-triggered human anti-mycobacterial responses. Science 339, 1448–1453 (2013).
14. O’Connor, R. M. et al. Genome-wide expression profiling identifies type 1 interferon response pathways in active tuberculosis. PLoS ONE 7, e45839 (2012).
15. Broom, C. I. et al. Detectable changes in the blood transcriptome are present after two weeks of antituberculosis therapy. PLoS ONE 7, e46191 (2012).
16. Broom, C. I. et al. Transcriptional blood signatures distinguish pulmonary tuberculosis, pulmonary sarcoidosis, pneumonias and lung cancers. PLoS ONE 8, e5630 (2013).
17. WHO. Treatment of Tuberculosis: Guidelines 4th edn (World Health Organization, Geneva, 2009) (WHO/HTM/TB/2009.420). http://www.who.int/tb/publications/tb_treatmentguidelines/en/.
18. Zhang, G. et al. A functional single-nucleotide polymorphism in the promoter of the gene encoding interleukin 6 is associated with susceptibility to tuberculosis. J. Infect. Dis. 205, 1697–1704 (2012).
19. Xu, H. et al. Pathway analyses identify novel variants in the WNT signaling pathway associated with tuberculosis in Chinese population. Sci. Rep. 6, 28530 (2016).
20. Zhang, G. et al. IL6 gene allele-specific C/EBPalpha-binding activity affects the development of HBV infection through modulation of Th17/Treg balance. Genes Immune. 16, 528–535 (2015).
42. Wiens, K. E. & Ernst, J. D. The mechanism for type I interferon induction by...

43. Mayer-Barber, K. D. et al. Host-directed therapy of tuberculosis based on...

26. Marie, I., Durbin, J. E. & Levy, D. E. Differential viral induction of distinct...

46. Chinese Society of Hepatology, Chinese Medical Association; Chinese Society...

35. Aucan, C. et al. Interferon-alpha receptor-1 (IFNAR1) variants are associated...

30. Bazan, J. F. Structural design and molecular evolution of a cytokine receptor...

27. Tailor, P. et al. The feedback phase of type I interferon induction in dendritic...

25. Sawyer, P. J. et al. Interferon regulatory factor 8-induced IL-6-mediated secretion and contributes to pathogenesis.

24. Mayer-Barber, K. D. et al. Host-directed therapy of tuberculosis based on...

23. Tailor, P. et al. The feedback phase of type I interferon induction in dendritic...

22. Mayer-Barber, K. D. et al. Host-directed therapy of tuberculosis based on...

21. Novikov, A. et al. Mycobacterium tuberculosis triggers host type I IFN signaling to regulate IL-1beta production in human macrophages.

20. Wiens, K. E. & Ernst, J. D. The mechanism for type I interferon induction by...

19. Mayer-Barber, K. D. et al. Host-directed therapy of tuberculosis based on...

18. Zhang, G. et al. An SNP selection strategy identified IL-22 associating with susceptibility to tuberculosis in Chinese. Sci. Rep. 1, 20 (2011).

17. Pigainers, R. A. et al. Suppressor of cytokine signaling (SOCS) 1 inhibits type I interferon (IFN) signaling via the interferon alpha receptor (IFNAR)-associated tyrosine kinase Tyk2. J. Biol. Chem. 286, 33811–33818 (2011).

16. Chinese Society of Hepatology, Chinese Medical Association; Chinese Society...

15. The feedback phase of type I interferon induction in dendritic cells requires interferon regulatory factor 8. Immunity 27, 228–239 (2007).

14. Feng, J. et al. Allele-specific induction of IL-1beta expression by C/EBBetapu and PU.1 contributes to increased tuberculosis susceptibility. PLoS Pathog. 10, https://doi.org/10.1371/journal.ppat.1004426 (2014).

13. Chen, X. et al. Diagnosis of active tuberculosis in China using an in-house gamma interferon enzyme-linked immunospot assay. Clin. Vaccin. Immunol. 16, 879–884 (2009).

12. Wiens, K. E. & Ernst, J. D. The mechanism for type I interferon induction by...

11. Mayer-Barber, K. D. et al. Host-directed therapy of tuberculosis based on...

10. Zhang, G. et al. Allele-specific induction of IL-1beta expression by C/EBBetapu and PU.1 contributes to increased tuberculosis susceptibility. PLoS Pathog. 10, https://doi.org/10.1371/journal.ppat.1004426 (2014).

9. Shen, Z. et al. Exhaustive genotyping of the interferon alpha receptor 1 chromosome 21q22.1 malaria susceptibility locus. PLoS Negl. Trop. Dis. 11, e0005308 (2017).

8. Novikov, A. et al. Mycobacterium tuberculosis triggers host type I IFN signaling to regulate IL-1beta production in human macrophages. J. Immunol. 187, 2540–2547 (2011).

7. Wiens, K. E. & Ernst, J. D. The mechanism for type I interferon induction by Mycobacterium tuberculosis is bacterial strain-dependent. PLoS Pathog. 12, e1005809 (2016).

6. Mayer-Barber, K. D. et al. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. Nature 511, 99–103 (2014).

5. Zhang, G. et al. Allele-specific induction of IL-1beta expression by C/EBBetapu and PU.1 contributes to increased tuberculosis susceptibility. PLoS Pathog. 10, https://doi.org/10.1371/journal.ppat.1004426 (2014).

4. Chen, X. et al. Diagnosis of active tuberculosis in China using an in-house gamma interferon enzyme-linked immunospot assay. Clin. Vaccin. Immunol. 16, 879–884 (2009).

3. Chinese Society of Hepatology, Chinese Medical Association; Chinese Society of Infectious Diseases, Chinese Medical Association. Guideline on prevention and treatment of chronic hepatitis B in China (2005). Chinese Med. J. 120, 2159–2173 (2007).

2. Mayer-Barber, K. D. et al. Host-directed therapy of tuberculosis based on...

1. Wiens, K. E. & Ernst, J. D. The mechanism for type I interferon induction by...