Figures and figure supplements

Rare missense variants in the human cytosolic antibody receptor preserve antiviral function

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Figure 1. TRIM21 is a highly conserved gene. Conservation of TRIM21 compared to invariant housekeeping genes (HDACs and actins), and immune genes including CGAS, STING and related TRIMs, TRIM5 and TRIM20. Comparison of variants between genes is expressed as number of missense variants (MVs) per 100 residues to normalize for differences in gene length (A-B). Frequency of nonsynonymous variants (A) common variants (≥1%) and (B) rare variants (<1%). Error bars represent standard deviation. Data provided in Figure 1—source data 1.

DOI: https://doi.org/10.7554/eLife.48339.002
Figure 2. Domain location of TRIM21 missense variants. (A) Schematic of TRIM21 showing how variants are distributed amongst component domains. (B–D) Mapping of variants onto structures of each domain. (B) RING (blue) and B-Box (orange) variants are marked on the autoinhibited structure of the
Figure 2 continued

TRIM21 RING-B-Box domains (PDB 5OLM). The second copy of the B-Box has been omitted and the location of a bound E2 enzyme (yellow) has been included instead to indicate the location of this functional interface (based on superposition of TRIM25 RING: E2 structure (5FER)). (C) Coiled-coil domain (green) based on TRIM25 (PDB 4CFG). Location of TRIM21 residues are marked based on sequence alignment. (D) PRYSPRY domain (red) with bound IgG Fc (yellow) based on PDB 2IWG. Residues in brackets are not present in the structure.

DOI: https://doi.org/10.7554/eLife.48339.004
Figure 3. PRYSPRY missense variants maintain antibody binding. (A) Representative Isothermal titration calorimetry (ITC) trace of IgG Fc against WT TRIM21 PRYSPRY fitted to the one set of sites model. (B) Summary of PRYSPRY variant binding affinities to IgG Fc. Consistent with known binding mode, TRIM21 PRYSPRY binds IgG with a stoichiometry of 2:1 (Keeble et al., 2008). Data provided in Figure 3—source data 1.

DOI: https://doi.org/10.7554/eLife.48339.005
Figure 3—figure supplement 1. Representative ITC traces of IgG Fc titration against each PRYSPRY variant. (A) N297H. (B) A390V. (C) G440R. (D) F446I. (E) K455E. (F) Q470K. (G) G471R. (H) Buffer control. (I) Binding signature plot. In most experiments, 40 μM of IgG Fc was titrated against 8 μM of PRYSPRY except for the F446I (D) where the concentrations were halved. Data provided in Figure 3—source data 1.

DOI: https://doi.org/10.7554/eLife.48339.006
Figure 3—figure supplement 2. Purification of TRIM21 PRYSPRY protein. (A–E) Superdex 75 (HiLoad 16/60) size exclusion chromatograms for a selection of TRIM21 PRYSPRY variants. (A) WT. (B) N297H. (C) A390V. (D) G440R. (E) F446I. (F) Variant TRIM21 PRYSPRY proteins resolved by 4–12% SDS-PAGE developed in MES-SDS buffer and stained using InstantBlue.

DOI: https://doi.org/10.7554/eLife.48339.007
Figure 4. PRYSPRY variants have profound differences in intrinsic stability. (A) Differential scanning fluorimetry of PRYSPRY variants to determine their melting temperature (Tm) using changes in intrinsic tryptophan fluorescence that occur upon unfolding (first derivative of 330/350 nm ratio). (B) Differences between variants are plotted as a ΔTm with respect to wild-type. (C) Correlation between ΔΔG for stability of PRYSPRY variants with predicted degree of deleteriousness from various algorithms. Correlation and p-values were calculated using the Pearson correlation coefficient. Data provided in Figure 4—source data 1.
DOI: https://doi.org/10.7554/eLife.48339.009
Figure 5. A bespoke system for ectopic TRIM21 expression at endogenous levels. (A) Map of lentivector containing the endogenous 2 kb upstream promoter sequence of the human TRIM21 gene followed by the 5'UTR (Exons 1–2) and TRIM21 coding sequence (Exons 2–7). (B) Immunoblot of TRIM21 and COX IV (loading control) in WT, TRIM21 KO (KO) or lentivector reconstituted (Reconst.) 293Ts with or without interferon-alpha (IFN-α) pretreatment. (C) Histograms of mCherry fluorescence intensity in cells transduced with lentivector encoding mCherry-TRIM21 driven by SFFV (Viral; Orange) or native TRIM21 promoter (Blue). Untransduced TRIM21 KO 293Ts were used as negative control (Red). (D) Immunoblot of TRIM21 and COX IV (loading control) in lentivector reconstituted 293Ts expressing the indicated TRIM21 variant, with the variants grouped into their host domains.

DOI: https://doi.org/10.7554/eLife.48339.011
Figure 5—figure supplement 1. TRIM21 expression levels in reconstituted cell lines. (A–C) Immunoblot of TRIM21 and COX IV (loading control) in TRIM21 KO (KO), WT or native promoter lentivector reconstituted (Reconst.) cell lines (A) 293T, (B) Hela, (C) hTERT-RPE-1. (D) Quantification of TRIM21 variant expression in reconstituted 293Ts (from Figure 5D) normalized to wild-type expression level. (E) Correlation between intrinsic stability (Tm) and cellular expression levels of PRYSPRY variants using linear regression analysis in GraphPad Prism7 ($R^2 = 0.72$). Data provided in Figure 5—source data 1.

DOI: https://doi.org/10.7554/eLife.48339.012
Figure 5—figure supplement 2. Native promoter driven mCherry-TRIM21 colocalizes with antibody coated AdV5. (A) Confocal microscopy images staining for human IgG and mCherry in TRIM21 KO 293Ts reconstituted with mCherry-TRIM21 driven by the native TRIM21 promoter. The cells were

Zeng et al. eLife 2019;8:e48339. DOI: https://doi.org/10.7554/eLife.48339

12 of 20

Human Biology and Medicine | Microbiology and Infectious Disease
fixed 30 min after infection by AdV5 coated with either WT 9C12 or 9C12 with the H433A mutation in its Fc. (B) Quantification of colocalization between 9C12-AdV5 and mCherry-TRIM21 using the ComDet plugin in Fiji (Schindelin et al., 2012). (C) Quantification of the mean and median mCherry intensities at 9C12-AdV5 spots using the ComDet plugin in Fiji (Schindelin et al., 2012). Data provided in Figure 5—source data 1.

DOI: https://doi.org/10.7554/eLife.48339.013
Figure 6. Viral neutralization by TRIM21 natural variants. (A) Neutralization experiments were carried out in TRIM21 KO 293T cell lines stably reconstituted with TRIM21 variants expressed at endogenous levels. Each stable cell line was challenged with AdV5-GFP in the presence of the anti-hexon monoclonal antibody 9C12. The AdV5 vector contains a copy of the GFP gene and relative infection levels were quantified by flow cytometry and normalized to that of virus only condition. Data compiled from at least two independent experiments (mean ± SEM) and fitted to a one phase exponential decay. (B) Correlation of neutralization efficiency ($K_{\text{neut}}$, the exponential decay constant calculated from (A)), with cellular protein expression levels (from Figure 5) or thermostability ($\Delta T_m$) using linear regression analysis in GraphPad Prism7. Variants are grouped into their host domains. The R234* variant was excluded from correlative analysis. Data provided in Figure 6—source data 1.

DOI: https://doi.org/10.7554/eLife.48339.015
Figure 6—figure supplement 1. F446I but not R234* can mediate viral neutralization with IFN-α priming. (A) Immunoblot of TRIM21 and COX IV (loading control) in lentivector reconstituted 293T cells expressing the indicated TRIM21 variant with or without IFNα pre-treatment. (B) Neutralization of AdV5-GFP in reconstituted 293T cell lines in the presence of anti-hexon 9C12 IgG. Relative infection levels were quantified by flow cytometry and normalized that of virus only condition. Data compiled from three independent experiments (mean ± SEM). (C) Percentage of infected cells in the neutralization experiment (B) in the absence of antibody. Data provided in Figure 6—source data 1.

DOI: https://doi.org/10.7554/eLife.48339.016
Figure 7. Unstable TRIM21 PRYSPRY variants function better at 33°C but lost more activity at 39.5°C. (A) Neutralization of AdV5-GFP in reconstituted 293T cell lines in the presence of anti-hexon 9C12 IgG at the indicated incubation temperatures. Relative infection levels were quantified by flow cytometry and normalized that of virus only condition. Data compiled from two independent experiments (mean ± SEM). (B) Immunoblot of TRIM21 and COX IV (loading control) in lentivector reconstituted 293T cells expressing the indicated TRIM21 variant after 24 hr incubation at the indicated temperatures. Data provided in Figure 7—source data 1.

DOI: https://doi.org/10.7554/eLife.48339.018
Figure 8. Viral sensing of by natural TRIM21 variants. (A) Stable 293T cell lines expressing TRIM21 variants were infected with AdV5 in the presence of anti-hexon 9C12 antibody and immune activation was measured 6 hr post infection using an NF-κB luciferase reporter. Data compiled from at least two independent experiments and expressed as fold change over that of virus only condition. EV (empty vector); mean ± SEM. (B) Correlation between NF-κB induction and cellular expression levels or thermostability by domain using linear regression analysis in GraphPad Prism7. The R234* variant was excluded from correlative analysis. Data provided in Figure 8—source data 1.

DOI: https://doi.org/10.7554/eLife.48339.020
Figure 9. Natural missense variants do not exert dominant negative effect. (A–D) Immunoblot (IB) for TRIM21 and COX IV (loading control) in (A) selected LCLs (B) Transduced WT 293T cell lines stably expressing TRIM21 variants under the native TRIM21 promoter. (C–D) CRISPR gene-edited 293T clones expressing the R234* and R118Q variant respectively. (E–H) AdV neutralization in the presence of anti-adenovirus hexon monoclonal IgG 9C12 in (E) selected LCLs. (F) WT 293T cells expressing the R234* and F446I variant. (G–H) CRISPR gene-edited 293T clones. Relative infection levels quantified by flow cytometry and normalized to virus only condition. Data compiled from at least two independent experiments (mean ± SEM). (I–L) Activation of NF-κB signaling by 9C12 coated AdV5 (I–J) or human TNF-α (K–L) in the respective cell lines. Data compiled from two independent experiments (mean ± SEM). Data provided in Figure 9—source data 1.

DOI: https://doi.org/10.7554/eLife.48339.022
Figure 9—figure supplement 1. Sanger sequencing chromatograms of the TRIM21 gene in LCLs. Codons with heterozygous mutation are highlighted in blue.

DOI: https://doi.org/10.7554/eLife.48339.023
Figure 10. Natural rare variants are less deleterious than expected by chance. The fraction of deleterious variants calculated using the indicated algorithms for all possible variants and naturally occurring variants that are present at a frequency of $\geq 10^{-5}$ or $<10^{-5}$.

DOI: https://doi.org/10.7554/eLife.48339.025