PPP2R2D Expression Quantitative Trait Locus Impacts Therapeutic Response to Anti-Thymocyte Globulin in Type 1 Diabetes

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Abstract

Background: Agents that deplete T cells, including low-dose anti-thymocyte globulin (LD-ATG), have shown success in preserving endogenous β-cell function in recent-onset type 1 diabetes (T1D). However, the degree of response to LD-ATG varies. While germline genetic polymorphisms are associated with many drug responses, the variants that correlate with LD-ATG response remain unknown.

Aims: 1) To identify genetic variants predictive of responsiveness to LD-ATG in the TrialNet ATG-GCSF Study, 2) to determine correlations between these genetic variants and immune phenotypes, and 3) to validate causal associations between response loci and effects on immune function.

Hypothesis: Genetic variants will predict responsiveness to LD-ATG by influencing immune function.

Methods: Genetic correlates of response were evaluated by performing a quantitative trait locus analysis for quantitative response (QR, observed minus expected C-peptide) and *ex vivo* immune phenotypes (n=28). CRISPR/Cas9-mediated knockout (KO) of the gene associated with the response locus was utilized to validate impacts on T cell exhaustion (n=6-8).

Results: We identified a locus adjacent to the Protein Phosphatase 2 Regulatory Subunit Bδ (*PPP2R2D*) gene that significantly associated with QR (p=7.70e-8). *PPP2R2D* encodes a regulatory subunit of PP2A, guiding recognition and dephosphorylation of substrates involved in various signaling pathways. T cell-specific *PPP2R2D* KO has previously been shown to promote murine CD8⁺ T cell exhaustion; thus, we hypothesized that variants limiting *PPP2R2D* expression may regulate human CD8⁺ T cell exhaustion. Subjects with rs9419387 A>G, associated with improved QR and reduced *PPP2R2D* expression by memory CD8⁺ T cells (p=0.024), showed significantly higher percentages of exhausted CD8⁺ T cells at baseline (p=0.039). While *PPP2R2D* KO increased pS6 Ser235/236 and Ser230/244 expression in memory CD8⁺ T cells shortly after T cell receptor stimulation (p<0.032), after chronic *in vitro* activation, CD8⁺ T cells showed enhanced expression of the exhaustion marker PD-1 (p=0.023), as well as decreased proliferation (p=0.008) and production of several cytokines (p<0.031).

Conclusions: Together, our work suggests that genetic variants affecting *PPP2R2D* expression may influence response to LD-ATG via impacts on T cell exhaustion. These data may support a precision medicine approach to identify individuals with or at-risk for T1D who are likely to respond to LD-ATG therapy.

PPP2R2D Locus Identifies Drug Responders

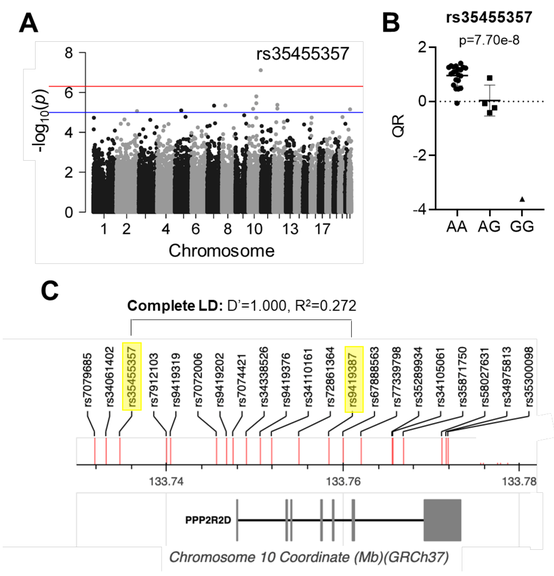

Figure 1. *PPP2R2D* (10q26.3) locus is associated with quantitative response (QR) to ATG in recent-onset T1D. (A) Manhattan plot of UFDIchip loci. Genome-wide significance (red line) at p = 5e-7 and suggestive association threshold (blue line) at p = 1e-5. **(B)** QR of ATG-treated subjects according to rs35455357 genotype, with unadjusted p-value from an additive linear model shown. N = 21 AA, 4 AG, 1 GG ATG-treated subjects of European ancestry. **(C)** Depiction of SNPs (highlighted yellow) in linkage disequilibrium with rs35455357, including the nearby expression quantitative locus (eQTL) for *PPP2R2D* in whole blood, rs9419387. LD statistics from 1000Genomes European ancestry population and gene diagram modified from LDlink.

Response Locus Impacts T Cell *PPP2R2D* Expression

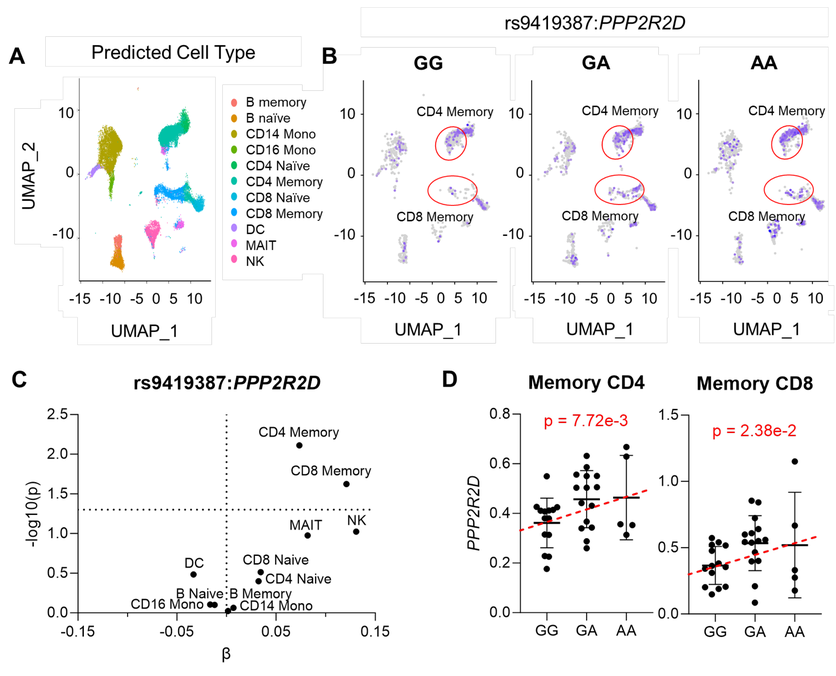

Figure 2. Response locus impacts T cell *PPP2R2D* expression. (A) Uniform manifold approximation and projection for dimension reduction (UMAP) of PBMC samples mapped to Azimuth reference, merged, and filtered on clusters present in all subjects. **(B)** Feature plots showing *PPP2R2D* mRNA expression in representative subject per rs9419387 genotype, downsampled to 2,000 cells each. Red circles = cell populations with significantly different expression of *PPP2R2D* by genotype. **(C)** Volcano plot of linear regression statistics from log-normalized average expression of *PPP2R2D* mRNA per cluster versus rs9419387 genotype. Analysis performed using matrixeQTL with age, gender, and sample batch as covariates. Horizontal dashed line at p = 0.05. **(D)** Average *PPP2R2D* expression in memory CD4⁺ or CD8⁺ T cells. N = 14 GG, 15 AG, 5 AA subjects.

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PPP2R2D Variants Associate with T Cell Exhaustion

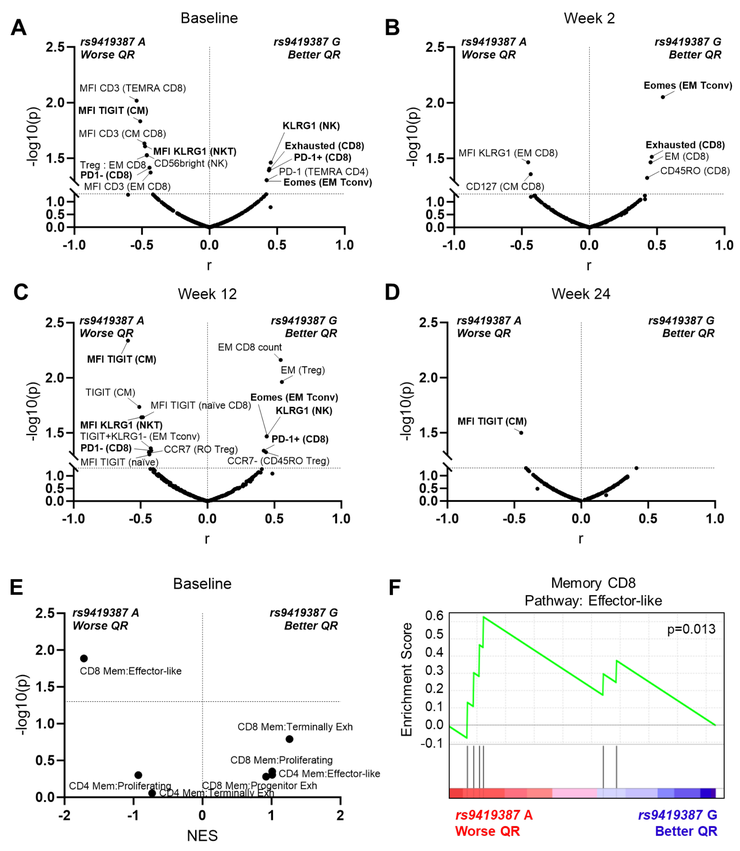

Figure 3. *PPP2R2D* variant associates with T cell exhaustion phenotypes. Volcano plots of Spearman correlation statistics for rs9419387 genotype, assuming additive effect, versus flow cytometry phenotypes from peripheral blood of ATG-treated clinical trial subjects at **(A)** baseline pre-treatment, **(B)** 2, **(C)** 12, and **(D)** 24 weeks post-treatment. Phenotypes increased in rs9419387 G subjects, associated with improved QR, shown on the right and phenotypes increased in rs9419387 A subjects, associated with poor QR, shown on the left side of each volcano plot. Significant phenotypes in multiple timepoints are bolded. N = 9 GG, 12 AG, 3 AA subjects. **(E)** Gene sets enriched in effector-like, proliferating, progenitor exhausted, and terminally exhausted T cell clusters derived from single-cell RNAseq of a viral model of T cell exhaustion were compared to naïve PBMC. Volcano plot of gene set enrichment analysis (GSEA) in memory CD4 or CD8 T cells, showing normalized effect size (NES) and -log(p-value) per comparison. Gene ranks derived from trans-eQTL analysis in matrixeQTL. Gene sets that were enriched in subjects carrying rs9419387 G are shown on the right and rs9419387 A on the left of the vertical dashed line. **(F)** Snapshot of gene set enrichment results for the effector-like pathway in memory CD8 T cells. **(G)** Violin plots of genes in the effector-like pathway that were significantly upregulated in memory CD8 T cells of rs9419387 A as compared to G-carrying subjects. N = 14 GG, 15 AG, 5 AA subjects.

PPP2R2D KO Enhances T cell Exhaustion

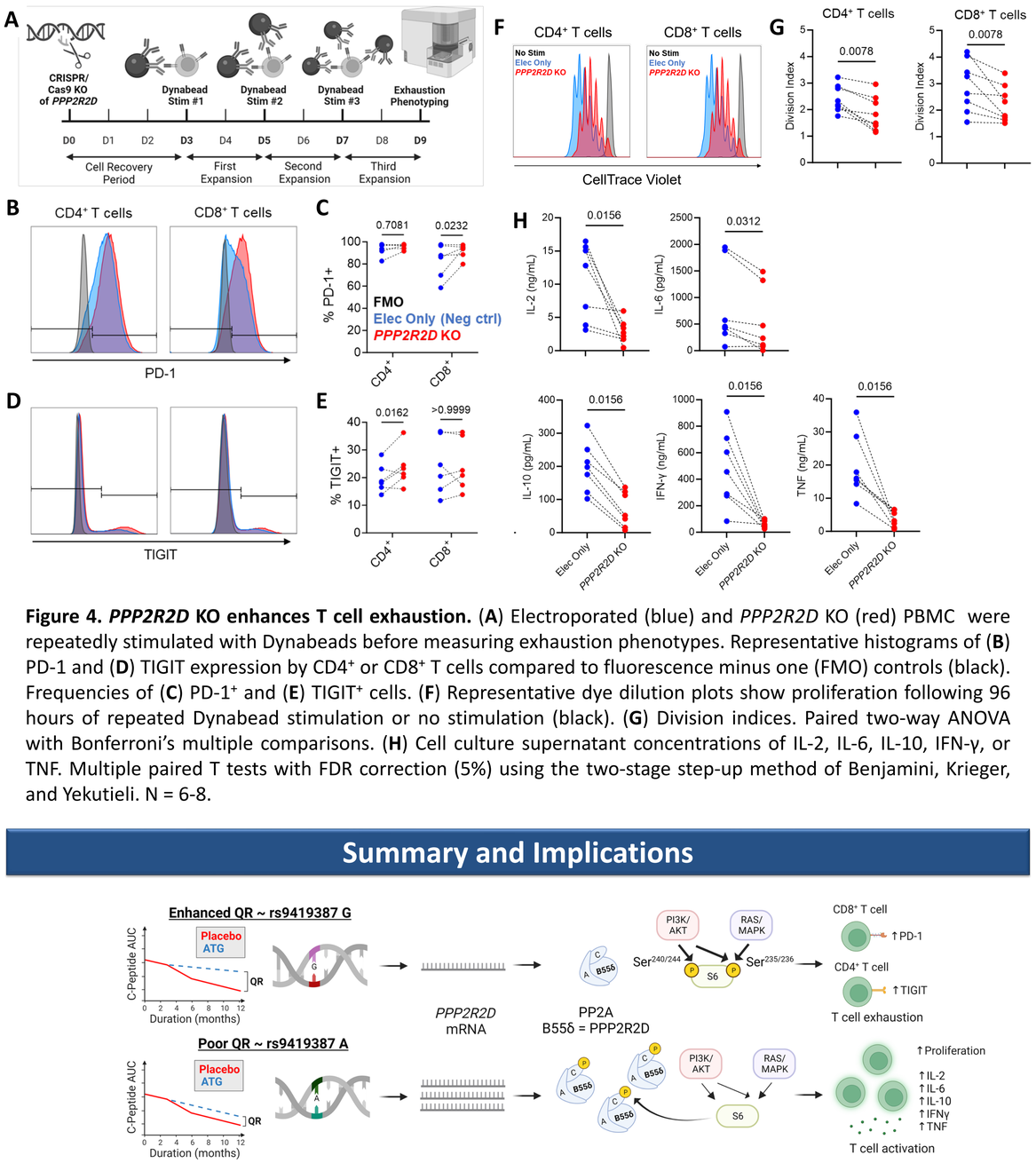

Figure 4. *PPP2R2D* KO enhances T cell exhaustion. (**A**) Electroporated (blue) and *PPP2R2D* KO (red) PBMC were repeatedly stimulated with Dynabeads before measuring exhaustion phenotypes. Representative histograms of (**B**) PD-1 and (**D**) TIGIT expression by CD4⁺ or CD8⁺ T cells compared to fluorescence minus one (FMO) controls (black). Frequencies of (**C**) PD-1⁺ and (**E**) TIGIT⁺ cells. (**F**) Representative dye dilution plots show proliferation following 96 hours of repeated Dynabead stimulation or no stimulation (black). (**G**) Division indices. Paired two-way ANOVA with Bonferroni's multiple comparisons. (**H**) Cell culture supernatant concentrations of IL-2, IL-6, IL-10, IFN-γ, or TNF. Multiple paired T tests with FDR correction (5%) using the two-stage step-up method of Benjamini, Krieger, and Yekutieli. N = 6-8.

Summary and Implications

- **Variant nearby *PPP2R2D* gene associates with LD-ATG response in T1D**
- **Response locus correlates with decreased *PPP2R2D* expression by memory T cells**
- **Response locus associates with increased T cell exhaustion *in vivo***
- ***PPP2R2D* knockout validates causal impact of PPP2R2D on T cell exhaustion *in vitro***
- **Future work required to determine whether differential drug dosing or drug selection would help to rescue response in PPP2R2D-mediated non-responders to ATG**