Genetic evidence for efficacy of targeting IL-2, IL-6, and TYK2 signaling in prevention of type 1 diabetes: A Mendelian randomization study

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Abstract

Aims

We aimed to investigate the genetic evidence to support the repurposing of drugs, already licensed or in clinical phases of development, for prevention of type 1 diabetes.

Methods

We obtained genome-wide association study (GWAS) summary statistics for the risk of type 1 diabetes, whole-blood gene expression, and serum protein levels, and investigated genetic polymorphisms near seven potential drug target genes. We used colocalization to examine whether the same genetic variants that are associated with type 1 diabetes risk were also associated with the relevant drug target genetic proxies, and Mendelian randomization to evaluate the direction and magnitude of the associations. Furthermore, we performed Mendelian randomization analysis restricted to functional variants within the drug target genes.

Results

Colocalization revealed that the blood interleukin (IL)-2 receptor subunit alpha (IL2RA) and, IL-6 receptor (IL6R), and IL-6 cytokine family signal transducer (IL6ST) gene expression levels shared the same causal variant with type 1 diabetes liability near the corresponding genes (posterior probabilities 100%, 96.3%, and 97.0%, respectively). Odds ratios (OR) of type 1 diabetes per 1-SD increase in the genetically proxied gene expression of IL2RA, IL6R, and IL6ST were 0.22 (95% confidence interval [CI] 0.17-0.27), 1.98 (95% CI 1.48-2.65), and 1.90 (95% CI 1.45-2.48), respectively. Using missense variants, genetically proxied tyrosine kinase 2 (TYK2) expression levels were associated with type 1 diabetes risk (OR 0.61, 95% CI [0.54-0.69]).

Interpretation

Our findings support the targeting of IL-2, IL-6 and TYK2 signaling in prevention of type 1 diabetes.

Research in context

What is already known?

- A recent large GWAS by Robertson et al. reported that 12 targets of drugs, which are already licensed or in in clinical development for other indications, were among the top 50 candidate targets for prevention of type 1 diabetes as judged by their priority index.

What is the key question?

- How strongly does the variability around these 12 drug target genes (IL2RA, IL2RB, IL2RG, IL6R, IL6ST, IL12B, IL23A, IFNAR2, JAK1, JAK2, JAK3, and TYK2) affect the risk of type 1 diabetes?

What are the new findings?

- When using rs61839660 near *IL2RA*, rs10908839 near *IL6R*, and rs7731626 near *IL6ST* as instruments for their respective gene expression, Mendelian randomization revealed that IL2RA expression is associated with decreased risk of type 1 diabetes (odds ratio [OR] 0.70 per 1 SD increase), while IL6R and IL6ST expression is associated with increased risk (OR 1.08 and 1.90 per 1 SD increase, respectively).
- When using a rare missense variant rs2304256 in *TYK2* as an instrument for TYK2 expression, Mendelian randomization indicated that an SD increase in TYK2 expression is associated with a decrease in the risk of type 1 diabetes (OR 0.61).

How might this impact on clinical practice in the foreseeable future?

- Our study suggests that drugs, such as aldesleukin, tocilizumab and olamkicept , which target IL-2 or IL-6 signaling and are already licensed for other indications, might be repurposed for prevention of type 1 diabetes.

Introduction

Type 1 diabetes is an autoimmune disease characterized by the loss of beta cell function. Despite advances in continuous glucose monitoring and insulin administration, managing type 1 diabetes remains a significant burden and few patients reach glycemic targets[1]. Several drugs have shown potential in delaying the loss in beta cell function in newly diagnosed cases[2], and teplizumab, a monoclonal anti-CD3 antibody, even delayed the onset of the clinical disease by an average of two years[3]. However, no current therapy can completely halt the disease progression, and therefore it is crucial to identify new efficacious drug targets.

Drug targets backed by genetic evidence have higher success rates in clinical development[4]. Genome-wide association studies (GWAS) enable the discovery of genomic regions strongly associated with the disease of interest. These associations can be considered as evidence for the involvement of the corresponding proteins in the disease pathogenesis, implying these proteins are potential drug targets for the disease. The evidence for a likely drug target can be further investigated via colocalization and Mendelian randomization. Colocalization can be utilized to study whether the same causal variant is shared between the drug target and the disease liability, or if the loci of risk allele and an allele influencing the drug target are distinct[5]. Furthermore, Mendelian randomization can be used to assess how much the genetic variability in drug target levels affects the risk of a disease in the population[6].

To inform the prioritization of targets for prevention of type 1 diabetes, we aimed to investigate the genetic evidence for efficacy of 12 drug targets in prevention of type 1 diabetes. These targets were selected because a prior GWAS reported that they are associated with the risk of type 1 diabetes and drugs that target them are already licensed for other indications than type 1 diabetes or they have progressed to clinical development (Supplementary Table 1).

Methods

Study design

We selected the drug targets based on a previous GWAS of type 1 diabetes by Robertson et al.[7] (Table 1; Supplementary Figure 1). Utilizing a priority index, the study ranked the drugs based on four factors including 1) existence of genetic variant(s) close to the potential target, 2) chromatin accessibility, 3) gene expression data in relevant cell types, and 4) protein-protein interactions[8]. We focused on 12 proteins (IL2RA, IL2RB, IL2RG, IL6R, IL6ST, IL12B, IL23A, IFNAR2, JAK1, JAK2, JAK3, and TYK2) which have already been targeted in clinical trials for autoimmune diseases and some of which have been licensed for other indications than type 1 diabetes (Supplementary Table 1)[7]. Among these targets, eight target genes (*IL2RA, IL2RB, IL6R, IL6ST, IL23A, JAK2, JAK3, TYK2*) had a locus associated with the risk of type 1 diabetes ($p<1\cdot10^{-5}$) within the distance of 1 million base pairs from the target gene (see Supplementary Figure 2). *IL23A* was excluded from the analyses because the data on its circulating levels were unavailable, leaving seven targets for the subsequent analyses. Genomic regions under investigation are listed in Table 1.

Table 1. Genomic regions under investigation

Gene	Chromosome	Start position[1]	End position[1]	Variants available[2]
IL2RA	10	6,010,689	6,062,367	9,683
IL2RB	22	37,118,666	37,175,118	5,489
IL23A	12	56,338,884	56,340,410	No pQTL data available
IL6R[3]	1	154,405,193	154,469,450	pQTL:8,116 eQTL: 4,274
IL6ST[3]	5	55,935,095	55,995,022	pQTL:12,024eQTL:4,253
JAK2	9	4,984,390	5,129,948	7,939
JAK3	19	17,824,780	17,848,071	7,142
TYK2	19	10,350,533	10,380,608	6,403

[1]Using genome build hg38.[2]Within ±1 million base pairs from the gene.[3]Both pQTL and eQTL data were analyzed because soluble and membrane-bound IL-6R and gp130 (encoded by IL6ST) have different biological actions (see Supplementary Figure 3).

All primary studies that generated the GWAS summary statistics used in our analysis have undergone institutional board review, received ethical approval, and conducted according to declaration of Helsinki [9–11].

Participants

We obtained the genome-wide association study (GWAS) summary statistics for type 1 diabetes, whole-blood gene expression (expression quantitative trait loci, eQTL) and serum protein levels (protein quantitative trait loci, pQTL) (Supplementary Table 2). eQTL data were used for loci near the all drug target genes since they code intracellular or membrane-bound proteins. However, pQTL data were also analyzed for loci in the vicinity of *IL6ST* and *IL6R* genes, since soluble forms of their respective proteins (gp130 and IL-6R, respectively) may also modulate IL-6 signaling (see Supplementary Figure 3) [12]. We included loci within 1 million base pairs from the seven potential drug target genes (Table 1). We obtained the data on type 1 diabetes risk variants from a subsequent GWAS of 18,942 cases and 501,638 controls of European ancestry from nine cohorts[9]. We obtained pQTL data for IL6ST from a GWAS of 35,559 Icelanders and eQTL data from GWAS of 31,684 individuals from 37 eQTLGen Consortium cohorts, most individuals being European[10, 11]. We have summarized the study population details of the utilized GWAS as well as methods of ascertainment of cases of type 1 diabetes, measurement of whole-blood gene expression, and serum protein levels in Supplementary note 1.

Colocalization

We conducted colocalization analysis to assess whether the genetic associations for type 1 diabetes risk near the seven drug target genes align with those for the whole-blood gene expression or serum protein levels of these targets. We performed a colocalization analysis using a 'coloc' package in R[5]. This method utilizes Bayesian principles to assess the relationship between two traits. It considers all variants within a specific genetic locus and evaluates the following hypotheses, assuming a maximum of one causal variant per trait:

H0: There is no association with either trait, implying no specific causal variants.

H1: There is an association with the exposure trait only.

H2: There is an association with the outcome trait only.

H3: There are associations with both exposure and outcome traits, two independent SNPs i.e., distinct casual variants.

H4: There are associations with both exposure and outcome traits, one shared causal variant.

A high posterior probability for H4 implies a shared causal variant for the two traits. A substantial posterior probability for H3 suggests the two traits are influenced by distinct causal variants linked to

each trait. We used the default prior probabilities of 10^{-4}, 10^{-4} and 10^{-5} for a variant being associated with the exposure trait, the outcome trait, and both traits, respectively.

Mendelian randomization

To evaluate the direction and magnitude of the causal effects, we performed Mendelian randomization for those variants that colocalized between drug target levels and the risk of type 1 diabetes (posterior probability for H4 >0.8). Mendelian randomization utilizes genetic variants to investigate the relationship between an exposure (e.g. drug target levels) and an outcome (e.g. type 1 diabetes risk) for causality. Under Mendel's law of assortment, genetic variants are accepted to be independent of other genetic alleles and can be used as valid instrumental variables to estimate the causal effect of the exposure on the outcome if the three assumptions are fulfilled:

1: the genetic variant is associated with the exposure,
2: the genetic variant is associated with the outcome only through the exposure, and
3: the genetic variant is not associated with any confounders[6].

Advantages of Mendelian randomization include limited susceptibility to reverse causation and confounding by external factors that influence both exposure and the outcome. Linkage disequilibrium (LD) may confound Mendelian randomization if genetic variants are not shared between the exposure and outcome, but they reside in the same genomic area. However, we investigated this possibility in the prior colocalization step.

Examination of functional variants near drug target genes

To further examine the potential causality of the putative drug targets on type 1 diabetes risk, we sought for functional missense variants in coding area of these seven drug target genes from Phenoscanner[13] that were associated with the protein/expression levels of the target at $p<10^{-5}$. We sought for missense variants only within the gene region for each gene. We used a more lenient threshold than the genome-wide significance of p<5e-8 since we only focused on cis-variants, and the threshold used can be interpreted as a Bonferroni-corrected threshold for 5,000 independent variants. These variants were then individually used as instruments in Mendelian randomization to test for causality of the targets on the risk of type 1 diabetes.

Tissue-specific gene expression analyses using bulk tissue and single-cell eQTL data

To further assess the tissue-specificity of the regions that showed evidence for association in Mendelian randomization, we conducted colocalization with tissue-specific gene expression and type 1 diabetes liability. We obtained data for *IL2RA*, *IL6R*, *IL6ST*, and *TYK2* single-cell eQTL of data for immune cell subsets from a GWAS of 982 individuals from OneK1K cohort and spleen and pancreas eQTL data from Genotype Tissue Expression (GTEx) project (v8) [14, 15].

Statistical analysis

All analyses were done with R version 4.2 (R Foundation for Statistical Computing, Vienna, Austria) using packages 'coloc', and 'TwoSampleMR'[5, 16]. Effect sizes for change in the risk of type 1 diabetes is reported per change in standard deviation of serum protein or mRNA levels, and the genetic variants were not weighted in any of the analyses. Beta and SE values for whole-blood RNA levels were calculated using formulae $\beta = z\left(\sqrt{2p(1-p)(n+z^2)}\right)^{-1}$ and $SE = 2p(1-p)(n+z^2)^{-1}$, where p = minor allele frequency, n=sample size, and z = z-score. Mendelian randomization estimates are reported as Wald estimates. To assess the instrument strength and potential weak instrument bias, we calculated the F-statistics for the instruments using formula $F=(\beta/\text{standard error})^2$.

We only used variants that were available in the GWAS summary statistics for both exposure and outcome traits within each genomic locus. All the LD r^2 values reported in this study were obtained from the European population of the 1000 Genomes project using the package 'ieugwasr'.The study protocol was not preregistered for this study. The code used to generate our results is available at https://github.com/jkoskenniemi/T1DSCREEN.

Results

The same genetic variants affect type 1 diabetes risk and whole-blood IL2RA, IL6R, and IL6ST gene expression

We found evidence for a shared causal variant between the risk of type 1 diabetes and whole-blood IL2RA (rs61839660, posterior probability 100%, Figure 1), IL6R (rs10908839, posterior probability 96.5%, Figure 2), and IL6ST gene expression (rs7731626, posterior probability 97.0%, Supplemental Figure 4). The variant rs10908839 is in strong LD ($r^2 = 0.76$) with the previously identified lead SNP rs2229238 associated with the risk of type 1 diabetes in the study by Robertson et al[7]. No evidence

for colocalization was observed between drug target levels and the risk of type 1 diabetes near the other target genes (Table 2, Supplementary Figures 5-10).

Table 2. Results of colocalization between drug target (eQTL/pQTL) and risk of type 1 diabetes.

Gene	**Trait**	**H0**	**H1**	**H2**	**H3**	**H4**
IL2RA	eQTL	0.0 %	0.0 %	0.0 %	0.0 %	**100.0 %**
IL2RB	eQTL	0.0 %	0.0 %	0.0 %	**99.7 %**	0.3 %
IL6R	eQTL	0.0 %	0.5 %	0.0 %	2.9 %	**96.5 %**
IL6R	pQTL	0.0 %	46.1 %	0.0 %	51.7 %	2.2 %
IL6ST	eQTL	0.0 %	0.3 %	0.0 %	2.8 **%**	**97.0 %**
IL6ST	pQTL	0.0 %	9.3 %	0.0 %	**90.5 %**	0.2 %
JAK2	eQTL	0.0 %	0.0 %	0.0 %	**100.0 %**	0.0 %
JAK3	eQTL	0.0 %	1.2 %	0.3 %	**98.5 %**	0.0 %
TYK2	eQTL	0.0 %	0.0 %	0.0 %	**86.6 %**	13.4 %

eQTL = expression quantitative trait loci, pQTL = protein quantitative trait loci. H0: no association with either trait, H1: an association with the exposure trait only, H2: an association with the outcome trait only, H3: associations with both exposure and outcome traits, two independent SNPs i.e., distinct causal variants. H4: associations with both exposure and outcome traits, one shared causal variant.

Genetically proxied variability in IL2RA, IL6R, and IL6ST expression is associated with the risk of type 1 diabetes

We investigated, using Mendelian randomization, the direction and magnitude of the expected change in the risk of type 1 diabetes if *IL2RA*, *IL6R*, and *IL6ST* were targeted, using variants identified as the most likely shared causal variant in colocalization (rs61839660 for IL2RA gene expression, F = 248; variant rs10908839 for IL6R gene expression, F = 148; and variant rs7731626 for IL6ST expression, F = 159). The results revealed an odds ratio (OR) of 0.22 (95% CI 0.17-0.27) on type 1 diabetes risk ($p=5.3 \cdot 10^{-43}$) per SD increase in genetically proxied IL2RA expression, OR of 1.98 (95% CI 1.48-2.65) per SD increase in genetically proxied IL6R expression ($p=5.2 \cdot 10^{-6}$) and OR of 1.90 (95% CI 1.45-2.48) per SD increase in genetically proxied IL6ST expression ($p=2.6 \cdot 10^{-6}$; Table 3).

Functional variant in coding area of TYK2 is associated with the risk of type 1 diabetes

We found 22 missense mutations in the examined drug target genes with GWAS summary data on type 1 diabetes risk (Supplementary Table 3). Most of the mutations had an allele frequency of <0.01 with notable exceptions of rs2228145 (MAF = 0.39) in *IL6R* as well as rs12720356 (MAF = 0.09) and rs2304256 (MAF=0.28) in *TYK2*. Summary statistics for both drug target level and type 1 diabetes risk were available for nine variants. One independent variant at LD $r^2<0.2$ within each locus (rs41316003 in *JAK2*, rs2304256 in *TYK2*, and rs141500365 *IL6ST*) was associated with the relevant drug target (protein or gene expression levels at $p<10^{-5}$). When using these variants as instruments in Mendelian randomization, the genetically proxied TYK2 gene expression was associated with the risk of type 1 diabetes (rs2304256: OR 0.61, 95% CI 0.54-0.69) whereas we found no clear evidence for association when using missense variants in *IL6ST* (OR 0.98, 95% CI 0.78-1.20), or *JAK2* (OR 0.74, 95% CI 0.46-1.20; Table 3). Supplementary Table 4 shows the LD matrix between the functional variants in coding area of *TYK2* as well as the lead SNPs associated with TYK2 expression and the risk of type 1 diabetes in the vicinity of *TYK2* gene.

Table 3. Mendelian randomization results for IL2RA, IL6R, IL6ST and TYK2.

Target	Lead variant identified in colocalization	MR OR (95% CI)	*p*-value	Lead functional variant	MR OR (95% CI)	*p*-value
IL2RA	rs61839660	0.22 (0.17-0.27)	$5.3 \cdot 10^{-43}$	-	-	-
IL6R	rs10908839	1.98 (1.48-2.65)	$5.2 \cdot 10^{-6}$	-	-	-
IL6ST	rs7731626	1.90 (1.45-2.48)	$2.6 \cdot 10^{-6}$	rs141500365	0.98 (0.78-1.20)	0.84
JAK2	-	-	-	rs41316003	0.74 (0.46-1.20)	0.22
TYK2	-	-	-	rs2304256	0.61 (0.54-0.69)	$1.4 \cdot 10^{-14}$

MR = Mendelian randomization, OR = odds ratio, CI = confidence interval.

Type 1 diabetes and IL2RA colocalizes in CD8+ effector memory T cells and type 1 diabetes and IL6ST in CD4+ and CD8+ naive and central memory T cells

To study which specific blood immune cells or tissues mediate the association between IL2RA, IL6R, IL6ST and TYK2 expression and type 1 diabetes risk, we analyzed their pairwise colocalization with type 1 diabetes risk in spleen, pancreas, and subsets of blood immune cells. IL2RA expression in CD8+ effector memory T cells colocalized (posterior probability 99.4%) with type 1 diabetes risk, with the lead causal variant rs61839660 being the same as for whole-blood IL2RA mRNA expression. Neither IL6R or TYK2 expression colocalized in any of available immune cells. However, IL6ST expression colocalized in CD4+ and CD8+ naive and central memory T cells (lead causal variant rs7731626; posterior probabilities 97.0%, and 92.3%, respectively), which also colocalized for whole-blood IL6ST expression. We did not observe such robust evidence for colocalization between *IL2RA, IL6R, IL6ST* or *TYK2* eQTL and the risk of type 1 diabetes in other cell types (Supplementary Table 5) or in spleen or pancreas (Supplementary Table 6).

Discussion

Leveraging data from large-scale GWAS and multiple QTL datasets, we investigated the genetic evidence for efficacy of seven candidate drugs in prevention of type 1 diabetes. Using colocalization and Mendelian randomization, we found genetic evidence to support the role of IL-2 and IL-6 signaling in the pathogenesis of type 1 diabetes. In addition, the investigation of functional missense variants suggested that TYK2 signaling is involved in the etiology of type 1 diabetes.

While the original GWAS of immune cell subsets did not report eQTL of *IL2RA* in regulatory T cells (Tregs), our evidence for the protective effect of blood IL2RA expression on the risk of type 1 diabetes could be interpreted to support the role of Tregs as a natural protection against type 1 diabetes. Low, but sufficient level of IL-2 is crucial for the survival and function of Tregs, which constitutively express IL2RA, IL2RB, and IL2RG to produce α-, β-, and γ-chains, respectively, required for trimeric high-affinity IL-2 receptor [17]. Naive T cells express IL2RB and IL2RG required for the intermediate affinity IL-2-receptor, but they express IL2RA only transiently when stimulated by antigen-presenting cells and are thus less stimulated by IL-2 when not activated. Thus, higher blood IL2RA expression could be a sign of increased quantity and function of Tregs, which maintain a level of tolerance towards self-peptides and decrease the risk of type 1 diabetes [18]. A previous small study showed that rs12722495, which is in strong LD (r^2 =0.89) with our lead *IL2RA* eQTL and type 1 diabetes risk locus rs61839660 near *IL2RA*, decreased IL2RA expression in Tregs as well as their sensitivity to IL-2 [19].

Alternatively, as IL-2 signaling increases the proliferation of the conventional T-cells and Tregs alike, IL-2 signaling might not decrease the risk of type 1 diabetes only by increasing tolerance, but also by promoting appropriate responses to pathogens. This is supported by our single cell level results, in which we observed evidence for colocalization between IL2RA expression and the risk of type 1 diabetes only in CD8+ central memory T cells (eQTL of Tregs were not available as they were not distinguished from other T cells). In a birth cohort study of children at high genetic risk of type 1 diabetes (TEDDY study), presence of enteroviral DNA in stool was associated with the risk of islet autoimmunity and the association was stronger in the case of persistent infections indicated by prolonged shedding of enteroviral DNA.[20] Likewise, children who developed islet autoimmunity presented longitudinal transcriptional signatures consistent with a less robust immune response against enteroviral infections than their matched controls [21]. Since the increased IL-2 signaling on CD8+ T cells during viral infections prioritizes robust immune response against production of long-lived memory cells, this explanation might also explain why rs61839660 near *IL2RA* strongly colocalized between the risk of type 1 diabetes and eQTL of *IL2RA* in effector memory T cells and CD8+ naive/central memory T cells.

The colocalization of IL2RA expression in CD8+ effector memory T cells with type 1 diabetes risk is further supported by the previous reports of IL-2 impairment leading to CD8+ T-cell exhaustion, potentially driven by both acute and chronic viral infections[22, 23]. Interestingly, our lead rs61839660 near *IL2RA*, which was associated with increased IL2RA expression and decreased risk of type 1 diabetes, was previously shown to be associated with higher risk of Crohn's disease [24]and lower risk of type 1 diabetes[25]. This suggests that the optimal balance between effector and regulatory T cell function may vary between autoimmune diseases [17]. Regardless of the possible mechanism, our finding supports the rationale of prevention trials of type 1 diabetes with low dose IL-2.

Consistent with our finding of protective effect of IL-2 signaling, we found that IL-6 signaling increased the risk of type 1 diabetes. IL-6 is secreted from macrophages rapidly in response to infections and tissue damage, which promotes various acute phase responses [29]. IL-6 signaling occurs as classic signaling through a cell membrane-bound IL-6R, *trans* signaling through soluble circulating IL6R and membrane-bound gp130 (encoded by IL6ST), and *trans* presentation by dendritic cells, in which IL-6 is presented to membrane-bound gp130 in T-cells via dendritic cell membrane-bound IL-6R (Supplementary Figure 3) [23]. IL-6 inhibits the development and function Tregs and promotes the development of pathogenic T-helper 17 (Th17) cells [30], which is inhibited by IL-2. Pronounced IL-6 signaling may alter the balance of Treg/Th17, which is a proposed causative factor in autoimmune diseases such as rheumatoid arthritis[30] and possibly also in type 1 diabetes [31].

While, IL-6 *trans* signaling and *trans* presentation may be a more potent inducer of autoimmunity than classic signaling[12], our finding that blood IL6R and IL6ST expression are associated with increased risk of type 1 diabetes may be explained by any of the three signaling modalities. However, since IL6ST expression in CD4+ and CD8+ naive or central memory T cells colocalized with type 1 diabetes and IL6R expression did not, it is tempting to speculate that *trans* signaling might be more important than classic IL-6 signaling in pathogenesis of type 1 diabetes.

In contrast to our findings, tocilizumab, a monoclonal antibody against IL6R, which blocks all three IL-6 signaling modalities, did not significantly affect the decline in residual beta cell function in individuals with newly diagnosed type 1 diabetes in a randomized, placebo-controlled, double-blind clinical trial[33]. However, this discrepancy may be partially explained by the timing of the intervention. Genetic polymorphisms typically exert life-long influence on risk of diseases including every stage of type 1 diabetes, whereas the intervention in the study by Greenbaum et al. took place after the diagnosis of type 1 diabetes, when a sharp fall in beta cell function had already taken place [34].

Whole-blood TYK2 expression and the risk of type 1 diabetes did not colocalize, whereas when using the missense mutation rs2304256 in *TYK2* as an instrument in Mendelian randomization, TYK2 expression was associated with type 1 diabetes risk. The absence of evidence in colocalization may reflect violations of the one-causal-variant assumption. Indeed, the missense mutation rs2304256 is in very high LD (r^2 =1.00) with the lead *TYK2* eQTL rs34725611. Moreover, rs2304256 is only in moderate LD (r^2 =0.10) with rs144309607, the lead variant on type 1 diabetes liability in colocalization, suggesting two independent signals. Of note, a known missense variant rs34536443 is not available in the eQTL data and therefore could not be used in colocalization. Despite the one-causal-variant assumption, 'coloc' is relatively robust to multiple causal variants, and colocalization methods allowing for multiple causal variants are highly sensitive to LD misspecifications in the reference panel[6]. Therefore, in the absence of an accurate LD reference, we proceeded with the 'coloc' method for our colocalization while acknowledging its limitations.

Overall, our *TYK2* findings are consistent with previous studies suggesting that TYK2 signaling is associated with the risk of type 1 diabetes[35]. Promising results have been found in clinical trials targeting TYK2 in autoimmune diseases supporting the potential of drug repurposing in type 1 diabetes[36]. Other reasons for the colocalization discordance could be that TYK2 is not activated until IFN alpha binding to IFNAR1 and *TYK2* RNA expressions are known to have low tissue and cell type specificity[37, 38].

It is important to note that Mendelian randomization estimates are only valid if the instrumental variable associations (relevance, independence, exclusion restriction) are met. The strong associations between the assessed genetic polymorphisms and the studied exposures suggested that the genetic instruments studied were relevant for the studied exposures. The strategy of selecting instruments from within the cis-region of the exposure of interest is an established approach for investigating drug effects[39]. C*is*-Mendelian randomization studies are by design less prone to horizontal pleiotropy and exposure misspecification (which may lead to violations of independence and exclusion restriction assumptions), as genetic variants typically exert the strongest influence on nearby genes and therefore most effects are secondary to reading of the nearby genes. However, restricting the instruments to cis-variants comes at the expense of potentially missing strong trans-variants that associate with the exposure. Furthermore, our colocalization results suggest that association between whole-blood IL2RA and IL6R gene expression and the risk of type 1 diabetes are unlikely to be caused by LD with a genetic variant that primarily influences the reading of other genes in the vicinity of *IL2RA* or *IL6R* loci.

Some aspects of generalizability of our results are also worth mentioning. While our study did not explicitly exclude individuals from non-European ancestries, the original GWAS studies primarily included individuals of European descent, which limits the spectrum of rare variants and the generalizability of our findings to other ancestries. Furthermore, our Mendelian randomization estimates represent the influence of small changes in IL2RA, IL6R and TYK2 gene expression during the entire life course before the diagnosis of type 1 diabetes. Therefore, these effect sizes cannot be directly extrapolated to clinical trials in which the doses are larger and exposures shorter and possibly outside a key sensitive window for disease development. Thus, natural history studies and clinical prevention trials should pinpoint the optimal stage of pathogenesis when to interfere with IL-2, IL-6, or TYK2 signaling to prevent type 1 diabetes. Finally, even if up to 50% of variability in genetic risk of type 1 diabetes is attributable to HLA-II locus[40], we could not analyze the interactions between SNPs reported here and the HLA genotype or genetic risk scores on risk of type 1 diabetes, as we did not have access to individual-level data.

In conclusion, our results provide genetic evidence that IL-2, IL-6, and TYK2 signaling are associated with type 1 diabetes risk. Our findings suggest that clinical trials investigating the efficacy of drugs such as tocilizumab (IL-6R antagonist that targets all IL-6 signaling modalities), olamkicept (soluble gp130Fc that blocks IL-6 trans signaling), and low dose aldesleukin (IL-2 analogue) may be promising candidates in prevention of type 1 diabetes.

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Declaration of interests

Authors declare no competing interests.

Author contributions

JK, VK, and DG designed the study. JK, VK, and JL curated data. TH and EK analyzed and visualized the data under supervision of JL, JK and VK. TH, EK, and JK wrote the first draft of the manuscript and all authors reviewed and edited the manuscript. All authors had access to data, and JK, VK, JL, TH, and EK accessed and verified the data. JK, VK, TH, and EK are guarantors for this article.

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Abbreviations

TYK2	tyrosine kinase 2
JAK1	Janus kinase 1
JAK2	Janus kinase 2
JAK3	Janus kinase 3
pQTL	protein quantitative trait locus / loci
eQTL	protein quantitative trait locus / loci
MAF	minor allele frequency
LD	linkage disequilibrium
GWAS	genome-wide association study
NK	natural killers
Treg	regulatory T-cell
Th17	T-helper 17

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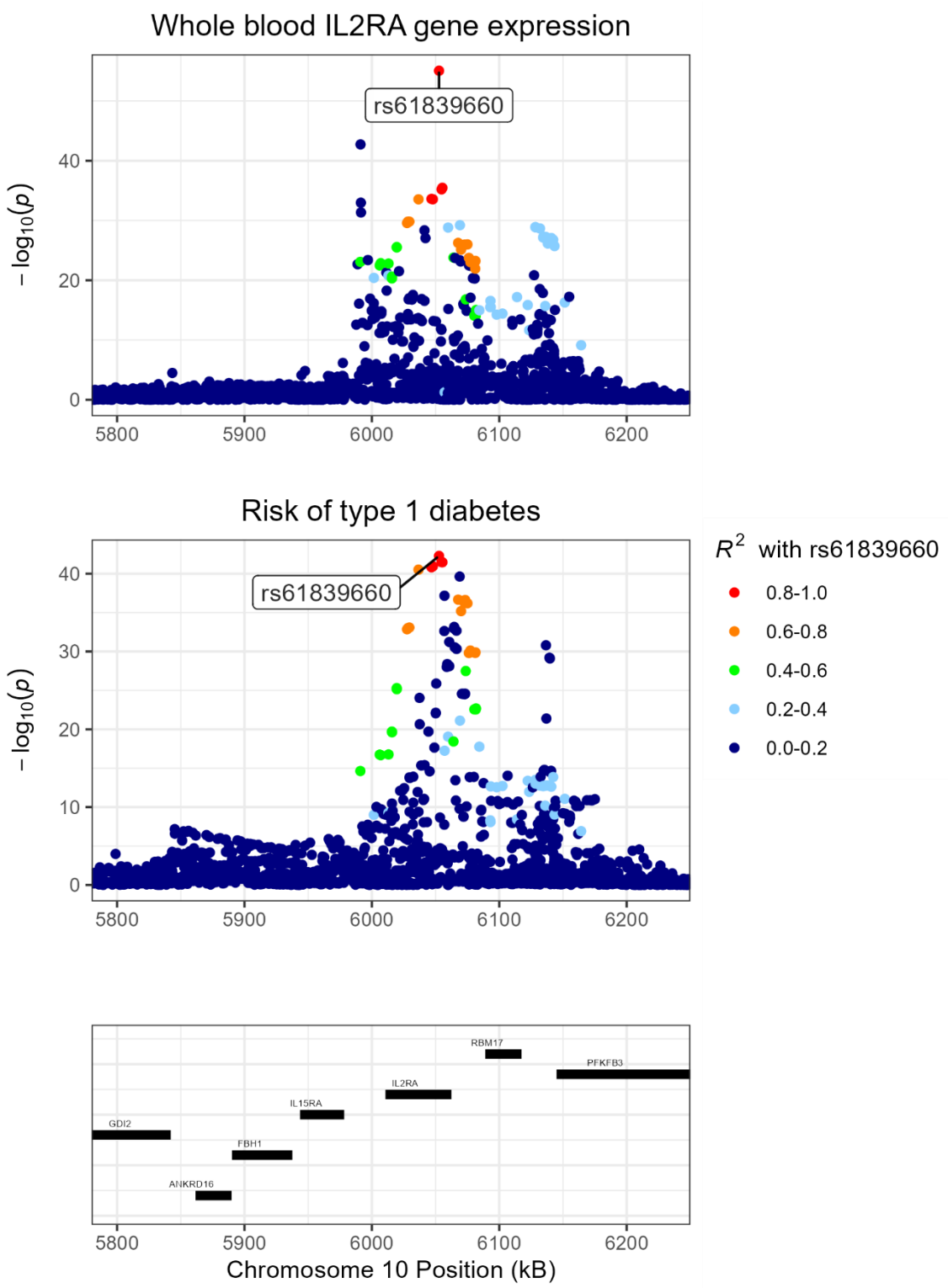

Figure 1. Regional Manhattan plot of IL2RA gene expression and risk of type 1 diabetes near *IL2RA* gene. The colours indicate the linkage disequilibrium (LD) r^2 value (based on 1000Genomes European reference) with rs61839660, the most likely shared causal variant identified in colocalization.

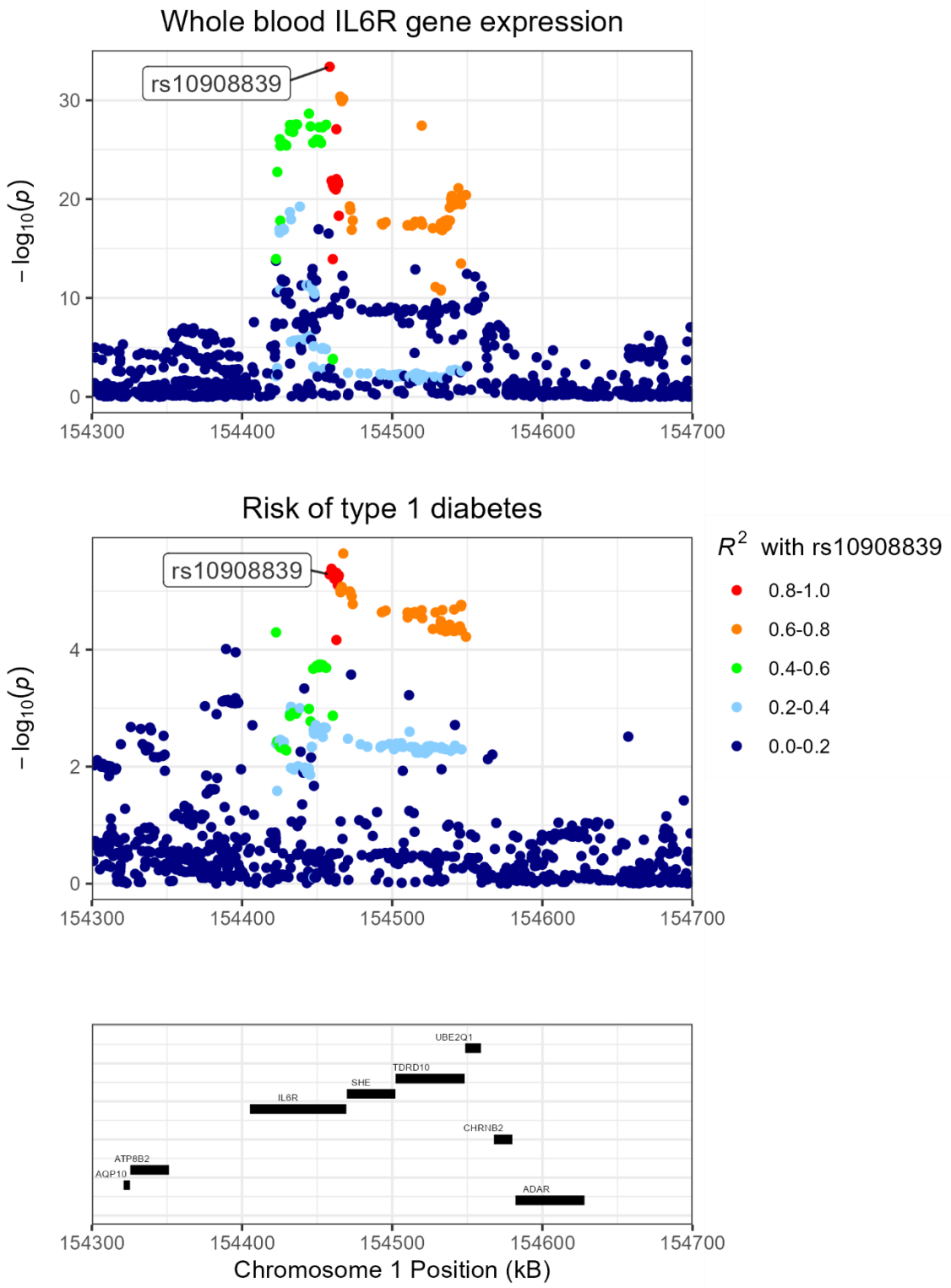

Figure 2. Regional Manhattan plot of IL6R gene expression and risk of type 1 diabetes near *IL6R* gene. The colours indicate the linkage disequilibrium (LD) r^2 value (based on 1000Genomes European reference) with rs10908839, the most likely shared causal variant identified in colocalization.