The Review of DIABETIC STUDIES

REVIEW

Chapter I.4

Protein Tyrosine Phosphatases and Type 1 Diabetes: Genetic and Functional Implications of PTPN2 and PTPN22

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■ Abstract

Protein tyrosine phosphatases (PTPs) play a central role in modulating the transduction of cellular signals, including the cells of the immune system. Several PTPs, *PTPN22*, *PTPN2*, and *UBASH3A*, have been associated with risk of type 1 diabetes (T1D) by genome wide association studies. Based on the current understanding of PTPs, it is clear that these variants impact antigen receptor signaling and cytokine signaling. This impact likely contributes to the development and progression of autoimmunity through multiple mechanisms, including failures of central and peripheral tolerance and the promotion of proinflammatory T cell responses. In this review, we discuss the genetic and functional implications of two of these PTPs, *PTPN22* and *PTPN2*, in the development of T1D. We describe the known roles of these proteins in immune function, and how the expression and function of these proteins is altered by the genetic variants associated with T1D. Yet, there are still controversies in the field that require further study and the development of new approaches to extend our understanding of these PTP variants, with the goal of using the information gained to improve our ability to predict and cure T1D.

Keywords: type 1 diabetes · genome-wide association study · PTPN22 · PTPN2 · LYP · TCPTP

1. Introduction

Genetic studies have led to the identification of over 50 genomic regions containing single nucleotide polymorphisms (SNPs) that are associated with type 1 diabetes (T1D) (www.t1dbase.org). Many of these genes participate in intracellular signaling, including a group of protein tyrosine phosphatases. Tyrosine phosphorylation plays a central role in the transduction and regulation of intracellular signals, including the regulation of antigen receptor signaling, cytokine-induced differentiation in lymphocytes, and insulin signaling. Protein tyrosine kinases (PTKs) potentiate the phosphorylation of tyrosine residues, while protein tyrosine phosphatases (PTPs) dephosphorylate tyrosine residues on proteins, thereby mediating positive or negative regulation of target pathways. Over 45 PTPs are expressed in lymphocytes, each having activities within signaling pathways. Therefore, alterations in the level of expression or function of PTPs have the potential to alter the function and fate of cells, including those involved in the immune response. Currently, three PTP genes have been associated with T1D, *PTPN22*, *PTPN2*, and *UBASH3A* [1]. Understanding the functional impact of these genetic variations in PTPs associated with T1D is likely to shed light on the mechanisms that drive disease development and assist in creating therapies to stop or reverse this process. In this review, we discuss variants of two PTPs which are associated with T1D, *PTPN22* and *PTPN2*, and their potential impact on disease.

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2. PTPN22

2.1 Genetic studies and broad association of PTPN22 1858T with autoimmunity

PTPN22 encodes the protein tyrosine phosphatase N22 (PTPN22), also referred to as lymphocyte tyrosine phosphatase (LYP) [2]. A single-nucleotide polymorphism (SNP) c.1858C>T (rs2476601) of *PTPN22* was found to be associated with T1D in 2004 [3]. Multiple subsequent studies have confirmed the association of this SNP with T1D, with a recent genome wide meta-analysis reporting a p-value of 5.93×10^{-80} and an odds ratio (OR) of 1.96 [4]. This variant is associated with multiple autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Graves' disease, and myasthenia gravis [5-8]. The 1858T variant is a missense mutation of the coding region, in which cytosine is replaced by thymidine at position 1858, resulting in a change from an arginine (R) at position 620 of the protein to tryptophan (W). The R620W variant is located within the P1 proline-rich repeat in the PTPN22 SH3 domain of the protein that mediates interaction with the C-terminal Src kinase (CSK), as described below, making it likely that this SNP is functionally significant. In addition to rs2476601, other coding variants within *PTPN22* have been identified in T1D subjects, indicating the importance of this gene in disease development [9].

2.2 Known function of lymphocyte tyrosine phosphatase (LYP)

LYP is expressed in all hematopoietic cells and is part of the Pro-Glu-Ser-Thr domain phosphatase (PEST) group of nonreceptor classical class I PTPs in human cell lines. LYP has been shown to be a negative regulator of T cell receptor (TCR) signal transduction via its interactions with the activating tyrosines of LCK (Y394), FYN (Y427), and zeta-chain-associated protein kinase 70 (ZAP70) as well as phospho-sites on TCRζ, CD3ε, VAV, and valosin-containing protein (VCP) [10-12]. Yu *et al*. have demonstrated that the phosphatase function of LYP is regulated by phosphorylation at Ser-35. The phosphorylation at this residue alters the conformational state of LYP and impairs its phosphatase activity resulting in an augmentation of TCR signal transduction [13]. The role of LYP as a regulator of TCR signaling is also bolstered by the development of a salicylic acid-based LYP inhibitor which binds to the catalytic site and enhances TCR signaling [13]. LYP has additional binding partners, including the adaptor molecule GRB2 (growth factor receptor-bound protein 2) and the E3 ligase c-CBL (Casitas B-lineage lymphoma proto-oncogene) [2], indicating that LYP may act alone or via interactions with other partners or substrates downstream of the TCR, B cell receptor (BCR), and other immune receptors.

Abbreviations:
BCL2 – B cell lymphoma 2
BCR – B cell receptor
BIM – BCL2-interacting mediator
C57BL/6 – inbred strain C57 black
c-CBL – Casitas B-lineage lymphoma proto-oncogene
cDNA – complementary DNA
CEU – Centre d'Etude du Polymorphisme Humain from Utah (Utah residents of northern European ancestry)
CLL - chronic lymphoid leukemia
CSF-1 - colony stimulating factor 1
CSK – C-terminal Src kinase
DAISY – Diabetes Autoimmunity Study in the Young
EGF – epidermal growth factor
ERK – extracellular signal-regulated kinase
GRB2 – growth factor receptor-bound protein 2
GWAS – genome-wide association study
HapMap – Haplotype Map (project)
HLA – human leukocyte antigen
IFNγ – interferon gamma
IFNGR2 – interferon gamma receptor 2
Ig – immunoglobulin
IL – interleukin
IRβ – insulin receptor beta
JAK – Janus kinase
LYP – lymphocyte tyrosine phosphatase
MAPK – mitogen-activated protein kinase
OR – odds ratio
PBMC – peripheral blood mononuclear cell
PEP – PEST domain phosphatase
PEST – Pro-Glu-Ser-Thr domain phosphatase
PI3K - phosphatidylinositol 3'-kinase
PLCγ2 - phospholipase Cγ2
PTK – protein tyrosine kinase
PTP – protein tyrosine phosphatase
PTPRC – protein tyrosine phosphatase, receptor type C
PTPN22 – protein tyrosine phosphatase, non-receptor type 22
RA – rheumatoid arthritis
SHIP - Src homology 2-containing inositol 5'-phosphatase
siRNA – small interfering RNA
SLE – systemic lupus erythematosus
SNP – single nucleotide polymorphism
STAT - signal transducer and activator of transcription
SYK – spleen tyrosine kinase
T1D – type 1 diabetes
Teff – effector T cell
TNFα – tumor necrosis factor alpha
Treg – regulatory T cell
TCPTP – T cell protein tyrosine phosphatase
TCR – T cell receptor
TRAF2 – TNF receptor-associated factor 2
VCP – valosin-containing protein
ZAP70 – zeta-chain-associated protein kinase 70

2.3 Dissecting the functional impact of LYP R620W on lymphocytes

Cell lines have been used to understand better the function of LYP in T cells. Studies in Jurkat cells have shown that LYP's inhibition of TCR signal transduction is enhanced when it interacts with the PTK CSK [10]. This interaction occurs in the region of LYP which includes amino acid residue 620. Owing to the position of the LYP risk variant within the P1 proline-rich region, which interacts with the SH3 domain of CSK, several groups have investigated the impact of the LYP R620W variant on LYP/CSK interaction. Jurkat cell lines transduced to express LYP 620W and CSK demonstrate a loss of interaction between LYP and CSK and an increase in TCR responses [14, 15]. However, when Jurkat cells are transduced to express LYP 620W alone, a blunting of TCR signal transduction is seen [16]. More recent studies have shown that LYP inhibits TCR-induced signaling after dissociation from CSK, and is recruited to lipid rafts; in this way a more rapid dissociation of the LYP-CSK complex could result in an increase in LYP 620W availability to lipid rafts and an increase in its inhibitory activity [17].

2.4 Function of the LYP R620W variant deduced from murine models of PEP and PEP R619W

Studies of the role of PEP (PEST domain phosphatase), the murine homologue of LYP, have shed light on its multiplicity of functions in the immune response. PEP and LYP share 89% identity at the PTP domain, and 61% identity in the non-catalytic domains. The majority of these studies have been performed in mice where the *Ptpn22* gene is knocked out [18-20], but more recently, a model in which *Ptpn22* is inducibly knocked down has also been described [21]. *Ptpn22* deficiency has been shown to enhance signaling through the TCR, as measured by calcium flux and phosphorylation of LCK, ZAP70, and extracellular signal-regulated kinase (ERK) (**Table 1**) [15, 18]. These alterations are most pronounced in memory T cells. They also result in increased proliferation of effector T cells and a general expansion of the T cell compartment over time. Regulatory T cells (Tregs) are also altered in these animals, with increased Treg number, IL-10 production, and suppressive function [19, 20]. *Ptpn22* deficiency has also been shown to cause an expansion of germinal centers and an increase of immunoglobulin production [18]. With respect to a disease phenotype, *Ptpn22*$^{-/-}$ effector T cells (Teffs) are more potent mediators of colitis upon cell transfer. However, these Teffs can be controlled by co-transfer of *Ptpn22*$^{-/-}$ Tregs (but not wild type Tregs) [20]. *Ptpn22*$^{-/-}$ mice are resistant to EAE [19] and NOD mice, in which *Ptpn22* is knocked down, have a decreased incidence of diabetes [21].

These studies indicate that PEP plays multiple roles in the murine immune response, likely regulating both the effector and regulatory T cell compartments. These findings are consistent with the role of the LYP R620W variant in human T1D, in that it alone is not sufficient to drive autoimmunity. Zikherman *et al*. explored the question of whether *Ptpn22*$^{-/-}$ mice would develop autoimmunity if additional factors that contribute to loss of tolerance are present. To this end, they crossed the *Ptpn22*$^{-/-}$ mouse onto a B6 mouse strain with the *Ptprc* (CD45) E613R mutation, a mutation known to cause a loss of B cell tolerance [15]. They observed that these animals had both enhanced T cell responses in the periphery and the thymus as well as an increase in B cell response to activation. Finally, the animals developed lupus-like disease with anti-nuclear antibodies, and lymphoproliferative disease.

These studies are informative as to the multiplicity of functions of this PTP in murine T cells, but limited in that they do not directly address the impact of the risk variant, which may lead to an alteration but not a loss of function. To address this question, Zhang *et al*. engineered a mouse to express the PEP R619W variant which is similar to the R620W variant found in human autoimmunity [22]. These animals did not develop autoimmunity, confirming a need for additional triggers in the process of autoimmunity. However, the animals had an increase in the number of dendritic cells, hyper-responsive T cell signaling and an increase in antibody production in response to vaccination. The authors attributed these changes to a relative decrease in expression of PEP 619W through enhanced calpain-mediated degradation of the PEP 619W protein. They concluded that in humans the likely functional implications of the variant was a loss-of-function of LYP owing to decreased protein levels in T and B cells. These findings contradict multiple studies in human B and T cells which find no difference in LYP protein based on the 1858T genotype. A second group has independently generated a PEP R619W knock-in-mouse and found normal stability of the PEP 619W protein. Like Zhang *et al*., they found en-

Table 1. Phenotypes associated with variants in the *PTPN22* gene in mouse models and human cells

Characteristic	Murine models			Human variants		
Variant type	$PEP^{-/-}$ or PEP_{KD}	$PEP^{-/-}CD45_{E613R}$	PEP619R	R263Q ↓ LYP phosphatase activity	LYP620W	CLL ↑ Lyp expression in CLL B cells
TCR signaling	↑ Signaling	↑ Signaling	↑ Signaling	↓ Signaling	↓ Ca+ flux, ↓ p-Tyrosine [25] ↑ p-ERK [22]	NA
Effector T cell	↑ Proliferation	↑ Proliferation	↑ Proliferation ↑ Memory pool		↓ IL-2 [16, 24] → Proliferation [25] ↑ Memory pool [25]	NA
Treg	↑ Number ↑ Suppression ↑ IL-10	ND			ND	NA
BCR signaling		↑ Signaling			↓Ca+ flux, ↓p-PLCγ2	↓p-LYN ⇒↑p-AKT
B cell development and maturation	↑ Germinal centers		↑ Transitional cells ↑ Germinal center B cell expansion		↑ Transitional ↑ B_{ND} ↓ BCR-mediated cell death [29]	↓ BCR-mediated cell death
Antibodies	↑ Ig production	ANA+	↑ Ig [22-23] ANA+ [23]		↑ Autoreactive BCR in the naïve B cell pool [30]	
Disease	↓ EAE in $PEP^{-/-}$ ↓ Diabetes in NOD PEP_{KD}	Lupus-like disease	None [22] Systemic autoimmunity, ↑ incidence of STZ-induced diabetes [23]	Q263 variant confers protection against human SLE	Associated withT1D, SLE, RA, Grave's disease [2-7]	CLL
References	[18-21]	[15]	[22-23]		[2-7, 16,22, 24-27, 29-30]	[28]

hanced antigen receptor signaling in lymphocytes, but in contrast to Zhang *et al*., loss of tolerance and autoimmunity have been observed [23].

2.5 Assessment of the role of LYP and the LYP R620W variant in human cells

The studies of cell lines and murine models have helped to define, at least in part, how PTPN22 participates in immune function in humans. However, these approaches have limitations due to the possibility that a cell line or an animal may not reflect the intracellular signaling environment or functional outcomes that are found in human cells. To fully understand how LYP and its variants impact immune function and human disease, studies with human lymphocytes have been performed.

Vang *et al*. were the first to examine the functional impact of LYP R620W on human T cells. They found that T cells from T1D subjects heterozygous for the 1858T allele, displayed decreased IL-2 production upon stimulation with anti-CD3/CD28 (**Table 1**) [16]. A second study with T1D 1858T carriers also found a decrease in T cell proliferation and IL-2 production upon CD3/CD28 stimulation [24]. In further studies, healthy individuals who carry the 1858T variant allele were shown to have an expanded CD4 memory compartment and a blunted response to stimulation

via CD3, as measured by Ca+ flux, IL-2, and IL-10 production, but no alteration in proliferation or production of the proinflammatory cytokines tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ) [25]. A decrease in IL-10 production was also described in 1858T carriers with ANCA vasculitis [26]. These studies seem to suggest that LYP R620W is a gain of function variant, leading to an increased inhibition of TCR signaling. Yet, the more recent study of the murine knock-in of PEP 619W by Zhang *et al*. suggested that the R>W substitution causes a loss of function due to decreased protein levels of PEP [22]. As part of that study, T cells from healthy and RA subjects were examined for LYP expression using a flow cytometric based assay. Using this approach, decreased expression of LYP was identified in individuals with the 1858T variant, and these subjects also had an increase in phosphorylation of ERK upon TCR stimulation [22]. Both the murine and human findings in this study seem to contradict the previous work using human peripheral blood mononuclear cells (PBMCs). The finding of decreased LYP expression in human lymphocytes has not been observed by other groups who have utilized Western blot, a more rigorous assay for protein levels [16, 27]. Also, phosphorylation of ERK has not been reported by other studies that have typically assessed more proximal aspects of TCR signaling in human samples. The differences between mouse and human primary T cells raise concerns about whether murine systems can sufficiently model LYP 620W function in human T cells.

LYP is also expressed in B cells. In chronic lymphoid leukemia (CLL), LYP is overexpressed resulting in a blunted BCR signal via spleen tyrosine kinase (SYK) which leads to a decrease in phosphorylation of phospholipase Cγ2 (PLCγ2), p38 MAPK, and ERK. However, the increase in LYP also leads to the inhibition of LYN and Src homology 2-containing inositol 5'-phosphatase (SHIP) which inhibit signaling via phosphatidylinositol 3'-kinase (PI3K) and AKT, leading to an increase in AKT activity [28]. This results in an enhancement of the prosurvival signals GSK3 (glycogen synthase kinase 3) and FOXO (forkhead box protein O), with the result that CLL cells escape BCR-mediated cell death [28]. LYP 620W has also been shown to impact primary B cells; expression of LYP is unaffected by the variant, but BCR signaling via PLCγ2 is decreased in naïve and memory B cells, suggesting a gain of function similar to that seen with overexpression of LYP in CLL [27, 29]. An extension of these studies to LYN, SHIP, and AKT has not yet been performed. However, studies have linked LYP 620W with alterations in B cell development and tolerance. It has been shown that such alterations can be associated with a resistance to BCR-mediated cell death, an expansion of transitional and anergic B cells, a decrease in circulating memory B cells, and an increase in the escape of autoreactive B cells into the transitional and mature naïve B cell compartment [29, 30]. These findings indicate that the LYP 620W variant likely contributes to the development of autoimmunity and autoantibodies via a B cell-intrinsic mechanism in addition to its impact on Teffs and Tregs.

2.6 Caveats related to studies of PTPN22 in human lymphocytes

The *in vitro* studies conducted in primary human lymphocytes have produced findings that partly differ from those conducted in immortalized cell lines or murine cells. Additional efforts are necessary to clarify the reasons for this discrepancy, to validate the present findings, and to reveal the true functions of PTPN22 in human lymphocytes. Human immune responses reflect a combination of genetic background, environment, and previous immune activation over years. Thus, the function of human primary lymphocytes not only reflects the direct impact of the variant itself, but may also be the result of compensatory mechanisms that have developed over time as a result of the presence of the genetic variant. In order to model this in animals, studies may need to follow up the animals as they age, and to determine impacts upon exposure to foreign antigens and pathogens. The influence of *PTPN22* c.1858C>T on the development of autoimmunity may in fact be due to both types of immune alterations, through genetic variation and compensatory mechanisms, as disease develops over time. Thus, both should be addressed as we move forward with studies of *PTPN22*.

2.7 LYP's role in the development of T1D

Studies that have assessed the impact of the *PTPN22* 1858T variant on disease development have indicated that carriers of the variant are younger at diagnosis of T1D [31]. In contrast, another study found the age at onset to be older among 1858T/T subjects [32]. In addition, *PTPN22* 1858T has been shown to be a predictor of more rapid progression to T1D among islet autoantibody-positive at-risk relatives [33]. Modeling of

known genetic factors has shown that combinations of genes which include *PTPN22* can be used to predict both islet autoantibody and diabetes development [34, 35]. Furthermore, the PTPN22 risk variant has been linked to the development of autoantibodies when combined with environmental triggers [36]. These studies support the concept that the *PTPN22* 1858T variant contributes to a failure in tolerance which leads to the development of autoantibodies and disease.

3. PTPN2

3.1 Genetic association of PTPN2 with autoimmunity

The *PTPN2* gene was first associated with autoimmunity in the Wellcome Trust Case Control Study in 2007 [37]; an SNP in the intergenic region 5.5kb downstream of the *PTPN2* gene, rs2542151, was found to be associated with susceptibility to T1D, Crohn's disease, and weakly with RA. This finding was replicated and refined by Todd *et al*. [38], who identified two non-coding SNPs in *PTPN2* that are independently associated with T1D: rs1893217 in intron 7 ($p = 1.16x10^{-11}$, OR = 1.3, 95% CI: 1.21-1.41) and rs478582 in intron 3 ($p = 9.15x10^{-9}$, OR = 0.82, 95% CI: 0.77-0.87). In the largest meta-analysis of T1D cases (11,781), control subjects (13,715), and family trios (4,342) to date, the association of *PTPN2* rs1893217 with T1D was highly significant ($p = 3.6x10^{-15}$) [1]. Note that rs1893217 in intron 7 is in complete linkage disequilibrium (LD, D'=1, r^2=1) with rs2542151 located 3' of the *PTPN2* gene, and thus their genotypes are interchangeable.

Like *PTPN22,* the *PTPN2* gene is associated with multiple autoimmune diseases, suggesting that it contributes to common mechanisms in the development and progression of autoimmune disease. Association of *PTPN2* rs1893217 has been confirmed for Crohn's disease ($p = 1.29x10^{-14}$, OR = 1.25, 95% CI: 1.18-1.32) [39], celiac disease ($p = 2.5x10^{-10}$, OR = 1.17, 95% CI: 1.12-1.23) [40], and, with weaker association, ulcerative colitis ($p = 4.78x10^{-5}$, OR = 1.12) [41] and RA ($p = 2.4x10^{-5}$) [42]. An initial association with Grave's disease [38] has not been replicated.

3.2 Known function of PTPN2

The *PTPN2* gene encodes a non-receptor protein tyrosine phosphatase and was originally cloned from a human T cell cDNA library, hence the alternative name T cell protein tyrosine phosphatase (TCPTP) [43]. While expression of PTPN2 is highest in cells of the hematopoietic lineage, it is expressed ubiquitously throughout the body and as early as day e8.5 of development in mouse embryos [43, 44]. Two isoforms of PTPN2 are expressed in human cells via alternative splicing; a 45kD variant (387 amino acids) which results from correct splicing of exon 9 to the terminal exon 10, and a 48kD variant which does not splice exon 9 to exon 10, but rather continues translation into intron 9, where a stop codon terminates the protein at amino acid 415 [43, 44]. The subcellular localizations of the 45kD and 48kD variants differ as a result of these alternative C-terminal sequences, and thus impact their substrate selection [45]. The 45kD variant is found primarily in the nucleus in resting cells due to a bipartite nuclear localization sequence, but can diffuse into the cytoplasm upon stimulation. In the cytoplasm, it can interact with cytoplasmic substrates and intracellular domains of transmembrane-bound receptors and their associated signaling proteins [46]. The 48kD variant contains a hydrophobic transmembrane domain and is targeted to the endoplasmic reticulum. In contrast to human cells, mouse cells express primarily the 45kD isoform of PTPN2 [47].

Structurally, the PTPN2 protein contains an N-terminal phosphatase domain and a C-terminal non-catalytic domain involved in autoregulation of catalytic activity and determination of subcellular localization, as described above [45, 48]. PTPN2 is closely related to the intracellular phosphatase PTP1B (PTPN1), with 74% identity in the catalytic domains [49]. Both PTPN2 and PTP1B prefer tandem phospho-tyrosine residues and share some substrates. Indeed, synthesis of inhibitors specific for each phosphatase has been challenging [50]. However, despite their relatedness, *Ptpn2* deficiency in mice results in defects in lymphopoiesis and systemic inflammation (discussed in detail below), whereas *Ptpn1* deficiency results in increased insulin sensitivity and protection from diet-induced obesity, reflecting their relative contributions to cytokine/antigen receptor signaling versus insulin receptor signaling [51].

Numerous substrates have been identified for PTPN2 that reveal roles in regulating signaling in response to growth factors, cytokines, hormones, and antigens. Specific targets have been identified through *in vitro* phosphatase assays and expression of substrate trapping mutants of PTPN2. Functional confirmation of substrates has been achieved using $Ptpn2^{+/+}$ and $Ptpn2^{-/-}$ fibroblasts and lymphocytes and PTPN2 siRNA in specific cell types. Using such techniques, PTPN2 has been

shown to dephosphorylate the epidermal growth factor (EGF) receptor and $p52^{Shc}$ in response to EGF, impacting association of $p52^{Shc}$ with GRB2 [52] and the colony stimulating factor 1 (CSF-1) receptor which mediates downstream ERK activation [53]. The Janus kinases JAK1 and JAK3 are dephosphorylated by PTPN2, resulting in modulation of IL-2, IFNγ, and IFNα signaling [54], while the transcription factors STAT1, STAT3, and STAT6 are targets of PTPN2 in response to IFNγ, IL-6, and IL-4, respectively [55-57]. TNF has been shown to stimulate PTPN2 interaction with the adaptor protein TNF receptor-associated factor 2 (TRAF2), with subsequent dephosphorylation of c-SRC and regulation of ERK activation [58]. Consistent with Src family kinases being substrates, PTPN2 was recently shown to dephosphorylate LCK and FYN following T cell receptor engagement, impacting activation of the downstream signaling molecules ZAP70, PLCγ1, and ERK [59].

PTPN2 has also been shown to play a role in regulating the response to the hormones prolactin, leptin, and insulin. The transcription factors STAT5A and STAT5B appear to be substrates for PTPN2 upon prolactin binding the prolactin receptor [60], and STAT3 is dephosphorylated by PTPN2 in response to leptin receptor engagement in the hypothalamus, attenuating leptin signaling [61]. In response to insulin, PTPN2 binds and dephosphorylates the insulin receptor beta (IRβ) in cooperation with PTP1B [47]. PTPN2 has been shown to control specifically the duration of downstream AKT phosphorylation, but not ERK phosphorylation, in PTP1B deficient and sufficient cells [47, 62]. Given the ubiquitous expression of PTPN2, it is likely that additional PTPN2 substrates will be identified that impact other pathways.

3.3 Functional impact of the PTPN2 risk variant

PTPN2 is the only gene within the T1D-associated LD block on chromosome 18, making it a likely candidate gene for the genetic association. No common coding variants in LD with either rs2542151 or rs1893217 have been identified, although rare coding changes in *PTPN2* have been identified through the 1000 Genomes Project (www.1000genomes.org). Thus, PTPN2 expression differences may contribute to T1D susceptibility. A modest but significant reduction in PTPN2 RNA levels with the rs1893217 risk allele have been observed in memory CD4 T cells from the peripheral blood of genotyped control subjects. This was confirmed in an independent data set generated from HapMap CEU EBV-transformed B cells [63]. However, neither rs1893217 nor rs2542151 overlap DNase I hypersensitivity sites, transcription factor binding sites, or microRNA target sites (genome.ucsc.edu), although binding sites for the vitamin D receptor have been reported in intron 7 near rs1893217 [64]. Thus, it seems likely that rs1893217 and rs2542151 are not directly responsible for alterations in PTPN2 expression, but may be found in LD with a variant that does alter expression.

3.4 Role of PTPN2 in autoimmunity deduced from murine models of PTPN2 deficiency

To understand better the role of PTPN2 *in vivo*, the *Ptpn2* gene has been disrupted globally in all mouse tissues of BALB/c and C57BL/6 mouse strains by homologous recombination [65] and Cre/LoxP recombination [66]. In all cases, *Ptpn2* deficiency results in Mendelian ratios of offspring that appear normal at birth. By 2 weeks of age, $Ptpn2^{-/-}$ mice begin to display growth retardation, and at 3-5 weeks of age, mice develop severe defects in B cell lymphopoiesis and erythropoiesis, resulting in death in 100% of animals. In contrast, mice heterozygous for the null allele display no obvious phenotype. On a BALB/c genetic background, $Ptpn2^{-/-}$ deficiency also results in a systemic inflammatory disease characterized by increased levels of IL-12p40, IFNγ, TNFα, iNOS, and mononuclear infiltrates in the spleen and non-lymphoid tissues [65, 67]. This inflammatory phenotype is not observed when *Ptpn2* deficiency is present on the more autoimmune resistant C57BL/6 genetic background [66].

Histologically, $Ptpn2^{-/-}$ mice are characterized by decreased bone marrow cellularity over time, with reduced numbers of progenitor B cells, pre B cells, immature IgM^+ B cells, and mature IgD^+ recirculating B cells, which is reflected in decreased peripheral B cell subsets [66, 68]. The thymus also has decreased cellularity over time due to a reduction in $CD4^+CD8^+$ double positive thymocytes, double negative thymocytes, and $CD4^+$ and $CD8^+$ single positive thymocytes, resulting in decreased numbers of $CD4^+$ and $CD8^+$ peripheral T cells [65, 66]. These B and T cell phenotypes result from both cell intrinsic and extrinsic factors, as evidenced by decreased B cell colony forming ability in $Ptpn2^{-/-}$ bone marrow compared to $Ptpn2^{+/+}$ bone marrow when grown on wild type stromal cells,

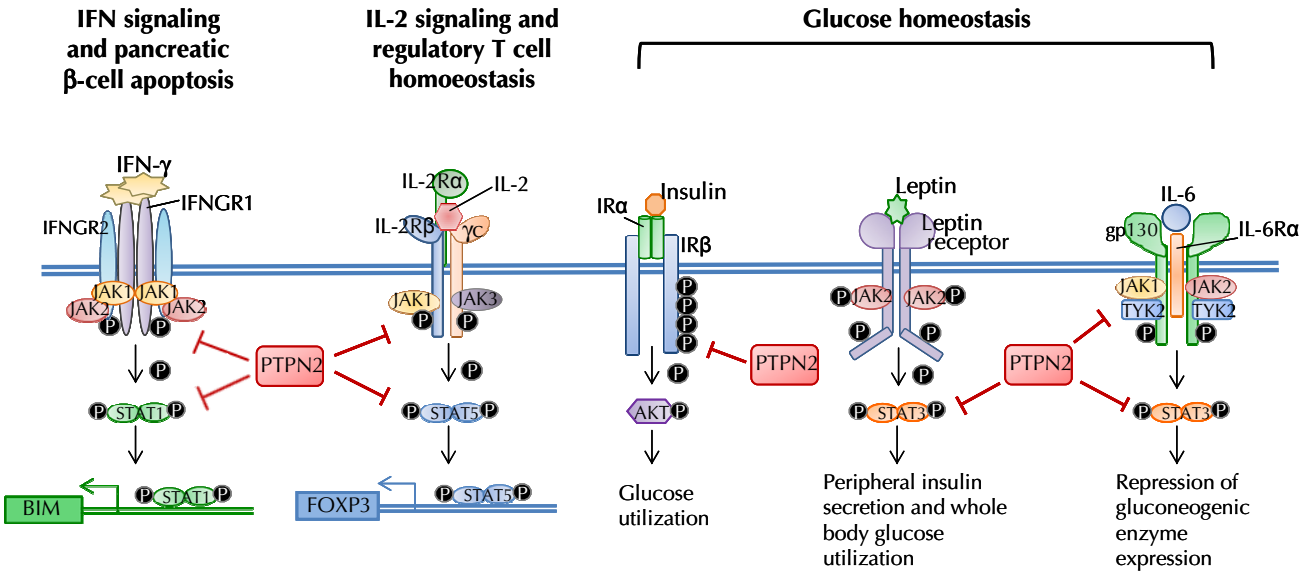

Figure 1. Potential roles for PTPN2 in the development of T1D. Experimental evidence indicates that PTPN2 regulates apoptosis of pancreatic β-cells upon exposure to inflammatory cytokines by modulating phosphorylation of STAT1, which induces the mitochondrial apoptotic pathway. The T1D-associated variant rs1893217 in the PTPN2 gene has been associated with reduced IL-2 receptor signaling in response to IL-2, impacting the maintenance of FOXP3$^+$ Tregs in T1D subjects. PTPN2 has also been shown to influence glucose homeostasis by dephosphorylating the insulin receptor β-chain in conjunction with the PTP1B phosphatase. In addition, PTPN2 impacts glucose levels by modulating STAT3 signaling downstream of the leptin receptor in the hypothalamus and in response to IL-6, repressing hepatic gluconeogenesis.

both of which are reduced when cultured on *Ptpn2*$^{-/-}$ stromal cells [68]. Extrinsically, cells in *Ptpn2*$^{-/-}$ mice are exposed to increased levels of inflammatory cytokines, while intrinsically, *Ptpn2* deficiency exacerbates signaling in response to growth factors, cytokines, and antigen receptor stimulation, since PTPN2 negatively regulates these pathways, as described above.

Recent experiments that specifically knocked out *Ptpn2* in CD4$^+$ T cells using a floxed *Ptpn2* locus and Lck-Cre mice have further defined the role of PTPN2 in T cell development, thymic selection, and response to antigen [59]. Consistent with LCK and FYN being direct targets of PTPN2, *Ptpn2*$^{fl/fl}$/Lck-Cre thymocytes and peripheral T cells display increased LCK and FYN phosphorylation, as well as increased phosphorylation of downstream TCR signaling pathway molecules following stimulation with anti-CD3. This translates into increased proliferation, and lowers the threshold for TCR-induced proliferation, antigen affinity, and need for costimulation via CD28 both *in vitro* and *in vivo*. The thymocytes of *Ptpn2*$^{fl/fl}$/Lck-Cre mice show evidence of increased positive selection, resulting in increased numbers of CD4$^+$ and CD8$^+$ thymocytes and peripheral T cells, particularly peripheral CD8$^+$ effector memory T cells. There is also increased responsiveness to IL-2 in *Ptpn2*$^{fl/fl}$/Lck-Cre T cells. Interestingly, at 48 weeks of age, *Ptpn2*$^{fl/fl}$/Lck-Cre mice develop inflammation and autoimmunity that is characterized by increased levels of circulating IL-6, TNF, and IFNγ, high levels of anti-nuclear antibodies, and infiltration of CD8$^+$ effector memory T cells in lymphoid and non-lymphoid tissues that can transfer disease.

Taken together, the phenotypes observed in *Ptpn2*$^{-/-}$ and *Ptpn2*$^{fl/fl}$/Lck-Cre mice provide clues to how decreased PTPN2 expression in humans may contribute to the loss of T cell tolerance and the development of inflammation, leading to autoimmunity. While conditional deletion of *Ptpn2* in T cells reveals T cell-intrinsic effects in the absence of non-T cell extrinsic factors, mice with global *Ptpn2* deficiency may model human PTPN2 deficiency more accurately since expression of PTPN2

is ubiquitous. Thus, cell-extrinsic effects of PTPN2 deficiency are likely to play a role in human disease settings, as they do in mouse models.

3.5 The role of PTPN2 in the development and progression of T1D

The role of PTPN2 in T1D is likely to be complex because of the ubiquitous expression of PTPN2 in both hematopoietic cells and non-hematopoietic cells, including β-cells in pancreatic islets. Moreover, based on *in vivo* mouse models, PTPN2 deficiency is likely to result in both cell-extrinsic and cell-intrinsic effects. Although the specific role of PTPN2 in T1D has only been studied in the last 5 years, pleiotropic functions are apparent, including effects on the maintenance of Tregs, β-cell apoptosis, and the response to insulin, as illustrated in **Figure 1**.

The presence of the risk genotype at *PTPN2* has been examined for its impact on the development of islet autoimmunity and T1D in several studies. A case-control study of European T1D cases reported that the frequency of carriers of the *PTPN2* rs2542151 G risk allele was significantly higher in T1D cases with an earlier onset (≤16y) compared with later onset T1D or controls [69]. This finding is not necessarily surprising since GWAS typically include T1D subjects with an age of onset <17y, which would enrich for genetic loci contributing to early onset disease. *PTPN2* rs1893217 risk genotype has also been studied in the BABYDIAB study in Europe, which has followed 1650 children of T1D parents, and the DAISY study in the US, following ~1700 children with a first-degree relative with T1D or with high-risk HLA DR3/4-DQ8 genotype [34, 35, 70, 71]. These children have been followed prospectively over time for the development of islet autoantibodies and progression to T1D. The advantage of these valuable cohorts is the ability to assess the predictive value of non-HLA genes to the initiation and progression of T1D. The disadvantage is that a small number of subjects in the cohort develop islet autoantibodies and progress to T1D, limiting the statistical power of the analysis. Nonetheless, Steck *et al*. reported a weak association of the *PTPN2* rs1893217 risk genotype with the development of islet autoimmunity, which remained significant after adjustment for family history and high-risk HLA genotype (p = 0.04, HR = 1.42, 95% CI: 1.02-1.99) [34]. Other studies have not detected a significant association of the *PTPN2* genotype with the development of islet autoantibodies [35, 70, 71]. No association of the *PTPN2* rs1893217 risk genotype with progression to T1D has been detected in these studies or others [34, 70-72], although an interaction between the *PTPN2* rs1893217 protective genotype and the vitamin D receptor SNP rs1544410 was associated with decreased risk of T1D in the DAISY cohort (p = 0.0004, HR = 0.24, 95% CI:0.11-0.53) [71]. Taken together, these studies indicate that the *PTPN2* rs1893217 genotype alone does not appear to be a significant risk factor for the development of T1D, but *PTPN2* may influence the development of islet autoimmunity and age at onset, suggesting a role for PTPN2 in disease initiation rather than progression.

Altered PTPN2 expression has been correlated with defects in IL-2 signaling and altered FOXP3 expression in Tregs in T1D. Consistent with JAK1/3 and STAT5 being substrates for PTPN2, the risk allele at the T1D-associated SNP *PTPN2* rs1893217 was correlated with decreased IL-2 signaling in peripheral $CD4^+$ T cells from genotyped control subjects (**Figure 1**) [63]. This phenotype was not due to altered expression of the IL-2 receptor, JAK1, JAK3, or STAT5; however, the rs1893217 genotype correlated with reduced expression of PTPN2 in $CD4^+$ memory cells, suggesting that PTPN2 may indirectly modulate IL-2 responsiveness in primary human T cells. In subjects with T1D, IL-2 signaling is further decreased compared with controls, indicating that the autoimmune environment acts coordinately with the PTPN2 genotype to reduce IL-2 signaling ([73] and unpublished data). Interestingly, PTPN2 expression was increased in T1D subjects compared with control subjects, most likely due to inflammatory cytokines which have been shown to induce PTPN2 expression [74, 75]. Functionally, diminished IL-2 signaling is associated with reduced maintenance of FOXP3 expression in natural Tregs from subjects with T1D and decreased FOXP3 expression in induced Tregs [73]. Thus, the effect of PTPN2 on IL-2 responsiveness may contribute to the development of T1D through an impact on Treg homeostasis, consistent with alterations in IL-2 and Tregs in NOD mice [76, 77]. Recent experiments have shown that the *PTPN2* rs1893217 risk variant acts independently of T1D risk variants in the *IL2RA* gene, which encodes the high-affinity IL-2 receptor, CD25, to reduce IL-2 responsiveness in T cells, potentially further impacting Treg persistence (unpublished data).

PTPN2 may also modulate pancreatic β-cell apoptosis in response to inflammatory cytokines.

Knockdown of PTPN2 expression in rat or human β-cells enhances apoptosis upon treatment with IFN-α, IFN-γ, IL-1β+IFN-γ, or TNF+IFN-γ (**Figure 1**) [74, 78]. Decreased PTPN2 expression is correlated with an increase in phosphorylation of STAT1 in rat insulinoma cells, and amelioration of STAT1 expression with a STAT1-specific siRNA protected cells from apoptosis. Thus, if the *PTPN2* rs1893217 risk genotype is associated with reduced PTPN2 expression in pancreatic β-cells, as found in lymphocytes, then it may predispose β-cells to apoptosis upon exposure to inflammatory cytokines via increased phosphorylation of STAT1.

Lastly, the role of PTPN2 in regulating insulin and leptin signaling may impact the development of hyperglycemia in T1D. The function of PTP1B and PTPN2 in these pathways has been a focus for type 2 diabetes (T2D) which is associated with the *PTPN1* gene [79-81]. Although the *PTPN2* gene is not associated with susceptibility to T2D, altered PTPN2 expression can impact hepatic gluconeogenesis. Heterozygous $Ptpn2^{+/-}$ mice fed a high fat diet display reduced fasting glucose levels compared with $Ptpn2^{+/+}$ littermates, which correlates with decreased fasting hepatic gluconeogenesis [82]. Decreased hepatic glucose output is driven by the mechanism of increased STAT3 phosphorylation downstream of IL-6 signaling in the liver, which suppresses expression of gluconeogenic enzymes (Figure 1). Peripheral insulin sensitivity and whole body glucose utilization is also impacted by PTPN2 expression via leptin signaling. Deletion of the *Ptpn2* gene specifically in neurons results in sustained STAT3 phosphorylation downstream of the leptin receptor in the hypothalamus of mice on a high fat diet, correlating with decreased diet-induced obesity, and reduced fasting plasma insulin and glucose [61]. These results suggest that decreased PTPN2 expression may protect from hyperglycemia by enhancing phosphorylation of STAT3 in response to IL-6 or leptin, whereas increased PTPN2 expression in the context of T1D could potentially contribute to increased glucose levels by attenuation of STAT3 phosphorylation.

4. Conclusions

The importance of the protein tyrosine phosphatases, PTPN2 and PTPN22, in T1D was first identified through genetic association studies. This has led to a series of further genetic and immunologic investigations which have enlightened us on the potential role of these proteins in the development and progression of T1D. In the case of each PTP, studies have uncovered alterations in multiple signaling pathways within multiple cell types caused by the genetic variants, leading to failures of central and peripheral tolerance, the promotion of proinflammatory T cell responses, and even the loss of regulation of glucose homeostasis. These findings have challenged our motivation to identify a single mechanism by which these variants lead to disease, or by extension a single pathway to target for therapeutic intervention. Further study is needed to determine if there is a single dominant immunologic alteration that contributes to disease. Alternatively, the study of these PTPs may demonstrate that the ubiquitous nature of these variants and their association with many autoimmune diseases is due, in part, to the multiplicity of ways in which these variants subtly alter the immune response.

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