Unraveling the functional implications of GWAS: how T cell protein tyrosine phosphatase drives autoimmune disease

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Genome-wide association studies (GWAS) have identified a large number of SNPs that are linked to human autoimmune diseases. However, the functional consequences of most of these genetic variations remain undefined. T cell protein tyrosine phosphatase (TCPTP, which is encoded by PTPN2) is a JAK/STAT and growth factor receptor phosphatase that has been linked to the pathogenesis of type 1 diabetes, rheumatoid arthritis, and Crohn’s disease by GWAS. In this issue of the JCI, Wiede and colleagues have generated a T cell–specific deletion of TCPTP and identified a novel role for this phosphatase as a negative regulator of TCR signaling. These data provide new insight as to how noncoding PTPN2 SNPs identified in GWAS could drive human autoimmune diseases.

Limitations of genome-wide association studies

Recent years have seen an explosion of genome-wide association studies (GWAS) designed to identify, in an unbiased manner, genetic loci associated with complex polygenic traits and diseases, including several autoimmune diseases (1). However, defining the functional consequences of disease-associated SNPs has been extremely challenging for several reasons. First, the majority of SNPs linked to polygenic autoimmune diseases individually confer a modest relative risk of developing disease (with odds ratios less than 1.5), suggesting that a complex genetic environment is required to manifest disease. Second, it is possible that some disease-associated SNPs are not causative, but instead act as genetic markers for rare disease-causing SNPs located nearby. Last, most disease-associated SNPs are noncoding, so it is likely that subtle and cell type–specific changes in transcript expression or splicing confer susceptibility to disease. Defining such subtle changes in gene expression has been difficult, and direct study of noncoding SNPs in model organisms or cell lines in order to define their effects on disease pathogenesis has not been possible.

Noncoding SNPs in and near the protein tyrosine phosphatase, non-receptor type 2 (PTPN2) gene have been associated with type 1 diabetes, Crohn’s disease, and rheumatoid arthritis (1–3). It is not well understood how these SNPs contribute to disease pathogenesis. In this issue of the JCI, Wiede and colleagues have generated mice with a T cell–specific deletion of Ptpn2 and used these animals to identify a novel role for the PTPN2 gene product T cell protein tyrosine phosphatase (TCPTP) as a negative regulator of TCR signaling (4). In doing so, they shed light on how disease-associated PTPN2 SNPs may drive human autoimmunity by dysregulating T cell development and homeostasis.

TCPTP is required to suppress systemic inflammation in mice

TCPTP is a ubiquitous non-receptor protein tyrosine phosphatase, with highest expression detectable in hematopoietic tissues and predominantly nuclear localization (2). Although TCPTP-deficient mice are born at Mendelian ratios, they become ill and die by three to five weeks of age (5). This early-onset disease is characterized by inflammatory infiltrates in multiple organs (6). Notably, these mice exhibit a dramatic increase in expression of proinflammatory cytokine transcripts in various tissues, including those encoding TNF-α, IFN-γ, and IL-12 (6). However, the site and stimulus for this increased cytokine production are unclear, as analysis of bone marrow chimeric mice indicates that TCPTP plays a critical regulatory role in both hematopoietic and non-hematopoietic cells (2). Studies of substrate-trap mutants and TCPTP-deficient cells have identified TCPTP as a negative regulator of multiple cytokine signaling pathways. JAK1, JAK3, STAT1, STAT3, and STAT5a/b are all putative TCPTP targets downstream of cytokines such as IL-2, IL-6, IL-4, and IFN-γ (2, 7, 8). In addition to JAK/STAT signaling pathways, TCPTP negatively regulates signaling downstream of several growth factor receptors, including the insulin receptor, EGFR, PDGFR, and CSF-1R (2). More recently, it has been shown that TCPTP negatively regulates TNF-α–induced ERK phosphorylation by targeting TNF receptor–associated factors (TRAFs) and Src family kinases (SFKs), substantially expanding the range of putative TCPTP substrates (9).

What do we know about the role of TCPTP in human autoimmune disease?

Modeling the functional consequences of noncoding disease-associated SNPs has been challenging. Direct study of primary cells from patients or healthy controls harboring disease-associated SNPs offers a powerful approach. Recently, Buckner and colleagues reported a modest (25%) reduction in expression of PTPN2 transcripts in primary human T cells harboring an intronic PTPN2 SNP associated with type 1 diabetes (10). They went on to probe the IL-2 signaling pathway and reported subtly impaired STAT5 phosphorylation in CD25+ regulatory T cells and CD45RO+ memory T cells bearing the disease-associated PTPN2 SNP. By contrast, it has been previously shown that TCPTP-deficient mouse T cells exhibit enhanced JAK/STAT phosphorylation upon IL-2 stimulation (7, 8). Reconciling these disparate results...
The damage and subsequent disease elicited by DSS is distinct from that observed in patients with Crohn’s disease, it may reasonably model how gut injury or impaired gut epithelial homeostasis interacts with commensal flora on a susceptible proinflammatory genetic background. Ptpn2–/+ mice may therefore prove to be a useful system to model how the Crohn’s disease-associated PTPN2 SNP drives disease.

Defining the function of TCPTP in T cells sheds light on the pathogenesis of human autoimmunity

The studies described in the previous section suggest how subtle alterations in TCPTP expression in T cells and other tissues could account for the disease association of noncoding SNPs in PTPN2. However, the precise function of TCPTP in T cells, key effectors in the pathogenesis of many autoimmune diseases, has not been well studied, in part because of the inflammation and mortality associated with global TCPTP deficiency in mice (5). In this issue of the JCI, Wiede and colleagues address this issue by generating mice in which Ptpn2 is conditionally deleted in T cells (4). Analysis of these mice has unmasked a cell-intrinsic function for TCPTP as a negative regulator of TCR signaling (Figure 1). Using substrate-trap mutants of TCPTP, Wiede and colleagues identified the T cell SFKs Lck and Fyn as direct substrates of TCPTP, although the mechanism by which the predominantly nuclear TCPTP is recruited to the membrane-associated SFKs remains to be determined. SFKs are critical mediators of TCR signaling and are themselves tightly regulated by inhibitory and activation loop tyrosine phosphorylation (12). By dephosphorylating the activation loop tyrosine of the SFKs Lck (Y394) (4).

Figure 1
Dual regulatory functions for TCPTP in T cells. TCPTP, the protein encoded by the PTPN2 gene, has previously been shown to target JAK1/3 and STAT5a/b downstream of IL-2 receptor signaling as well as other cytokine pathways. In this issue of the JCI, Wiede and colleagues identify a new role for TCPTP as a negative regulator of TCR signaling through its ability to dephosphorylate the activation loop tyrosine of the SFK Lck (Y394) (4).
of systemic autoimmunity, were unper-
turbed. Taken together, these observations 
of Wiede and colleagues suggest that an 
altered threshold for T cell activation may 
contribute to abnormal T cell homeosta-
sis in the T cell–specific TCPTP-deficient 
mice. However, TCPTP is a critical negative 
regulator of JAK/STAT signaling pathways 
downstream of cytokines such as IL-2, 
which has an important function in T cell 
activation (2, 7, 8). Therefore, it remains 
possible that abnormal T cell homeostasis 
in these mice is not driven exclusively by 
dysregulated TCR signaling (Figure 1).

Wiede and colleagues also demonstrat-
ed that conditional deletion of TCPTP in 
T cells was sufficient to drive an inflamma-
tory disease on the C57BL/6 background, 
with onset by one year of age (4). This dis-
ease occurs much later than in mice com-
pletely deficient in TCPTP (5), suggest-
ing that TCPTP plays a critical negative 
regulatory role in other cell types to sup-
press inflammation. The disease observed 
by Wiede and colleagues in mice with 
T cell–specific TCPTP deficiency (4) was 
characterized by increased serum levels of 
proinflammatory cytokines such as IL-6, 
TNF-α, and IFN-γ, as well as lung and liver 
immune cell infiltrates. In addition, these 
mice exhibited evidence of non-cell-autono-
myous dysregulation of B cell homeostasis, 
including autoantibody production and 
spontaneous germinal center formation. 
Whether the disease described by Wiede 
and colleagues is driven by dysregulation 
of TCR signaling exclusively, or may also 
be due to derepression of cytokine signal-
izing pathways in T cells, remains unclear. 
Because both T cell development and 
T cell function are affected in these mice, it 
is uncertain whether selection of an altered 
TCR repertoire during thymic develop-
ment or inappropriate peripheral T cell 
activation drives disease.

Dysregulated TCR signaling is 
implicated in human autoimmune 
disease

The conditional T cell deletion of Ptpn2 
reported by Wiede and colleagues (4) 
suggests that dysregulated TCR signaling 
may contribute to autoimmunity in patients 
harboring disease-associated PTPN2 SNPs 
(1–3). Although Wiede et al. did not directly 
probe this pathway in patient samples, the 
TCR signaling pathway has been previously 
implicated in autoimmune disease patho-
genesis by GWAS (13). A particularly well-
studied and relevant example is the PTPN22 
gene, which encodes Lyp (murine ortholog, 
Pep), a hematopoietically expressed cyto-
plasmic tyrosine phosphatase that is struc-
turally unrelated to TCPTP. A single cod-
ing SNP in this gene (PTPN22 C1858T) has 
been associated with multiple autoimmune 
diseases, including systemic lupus erythe-
matosus, rheumatoid arthritis, and type 1 
diabetes (1, 14–16). Lyp/Pep, like TCPTP, 
negatively regulates TCR signaling, at least 
in part by dephosphorylating the activa-
tion loop tyrosine of SFKs (17). Indeed, 
Pep-deficient mice recapitulate a subset of 
phenotypes identified by Wiede and 
colleagues in their T cell–specific TCPTP-
deficient mice (4), including enhanced 
thyminic positive selection, accumulation 
of effector/memory phenotype T cells, and 
enhanced spontaneous germinal center 
formation (18). However, the disease that 
develops spontaneously in the T cell–spe-
cific TCPTP-deficient mice does not occur 
in Pep-deficient mice. This may be due to 
the distinct potency and temporal require-
ment of the two phosphatases during TCR 
signaling. Alternatively, this may suggest 
that dysregulation of cytokine signaling 
pathways in T cell–specific TCPTP-defi-
cient mice contribute to late-onset inflam-
matory disease.

Unlike PTPN2 noncoding SNPs, the 
PTPN22 C1858T SNP results in a coding 
mutation (LypR620W) that has rendered 
it particularly amenable to study in model 
systems. It has been shown that this single 
residue change impairs constitutive asso-
ciation of Lyp with its binding partner c-Src 
tyrosine kinase (Csk) (16, 19). Csk serves to 
recruit Lyp to the proximity of its substrates 
and cooperates to inhibit TCR signaling by 
phosphorylating the inhibitory tyrosine 
of the SFKs (17). Although this mutation 
might thus be predicted to impair Lyp func-
tion, studies in cell lines and primary human 
cells have yielded conflicting results (19–22). 
Most recently, Zhang et al. have generated 
a knock-in mouse harboring the disease-
associated PTPN22 SNP (23). They show 
that T cells from these animals as well as 
from patients homozygous for the risk allele 
exhibit hyperresponsive TCR signaling that 
is at least partly due to degradation of the 
Lyp/Pep R620W variant. These data strongly 
suggest that the PTPN22 risk allele produces 
a hypomorph and may have functional con-
sequences similar to those of noncoding 
SNPs in PTPN2. However, as with PTPN2, 
function of the disease-associated PTPN22 
C1858T SNP in cell lineages other than 
T cells may also be important (21, 23).

Although the PTPN2 and PTPN22 SNPs 
may have similar functional consequences 
for TCR signaling and exhibit overlapping 
motor disease association for type 1 dia-
betes and rheumatoid arthritis, some impor-
tant differences stand out. In particular, the 
PTPN22 C1858T allele is associated with sys-
temic lupus erythematosus, but the PTPN2 
genic locus is not (15). Conversely, while the 
PTPN2 locus is linked to Crohn’s disease, 
the disease-associated PTPN22 C1858T 
allele subtly lowers risk for this condition 
(1, 24). This suggests that distinct pathways 
and/or distinct cell types may be regulated 
by the PTPN2 and PTPN22 SNPs.

Future directions

Mouse and human data suggest that 
reduced expression of TCPTP might drive 
human autoimmune disease by enhanc-
ing signaling downstream of the TCR, 
cytokines, or growth factors to produce a 
proinflammatory cytokine milieu (2, 4, 5, 
10, 11). Importantly, disease may reflect 
abnormalities in immune cell development 
as well as mature immune cell function. 
Because TCPTP is ubiquitously expressed 
and regulates multiple signaling pathways, 
dissecting the functional consequences of 
human SNPs in the gene encoding this 
protein will be challenging.

To clarify both the function of TCPTP 
in immune homeostasis and its role in 
human disease, several key questions 
remain to be addressed. The identifica-
tion of SFKs as substrates of TCPTP in 
T cells by Wiede and colleagues (4, 9) raises 
the possibility that TCPTP regulates SFK-
dependent signaling pathways in other cell 
types. To better clarify the contribution of 
the PTPN2 genetic locus to human auto-
immune diseases, extensive resequencing 
of PTPN2 to identify rare coding SNPs 
will be important, as will defining PTPN2 
transcript expression in human subjects 
harboring disease-associated SNPs. Fur-
ther evaluation of JAK/STAT, growth fac-
tor receptor, and SFK-dependent signal-
ning pathways in primary human cells may 
help define the functional consequences 
of these SNPs. Finally, modeling disease in 
mice will continue to be critical. Focus on 
heterozygous rather than knockout ani-
mals in the context of susceptible genetic 
backgrounds or environmental provoca-
tion will be informative. As this work pro-
ceeds, the mice bearing a floxed PTPN2 
allele generated by Wiede and colleagues 
and described in this issue of the JCI (4) 
will prove to be a very important tool.
A new medical therapy for Cushing disease? 

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Members of the ErbB family of cell surface tyrosine kinase receptors are important targets for cancer treatment because they frequently contribute to the pathogenesis of malignancy. In this issue of the JCI, Fukuoka et al. generate data that suggest that using a tyrosine kinase inhibitor (TKI) against epidermal growth factor receptor (EGFR; also known as ErbB1) may be a novel approach for treating patients with hypercortisolism due to pituitary corticotroph adenomas (Cushing disease). While surgical resection remains the cornerstone of treatment for individuals with such tumors, this study suggests that TKIs could perhaps be used to reduce tumor size prior to surgery or to treat recurrent disease after surgery.

Cushing disease is a condition in which the pituitary gland releases too much adrenocorticotrophic hormone (ACTH), the hormone that stimulates the secretion of cortisol from the adrenal cortex (Figure 1 and ref. 1). Cortisol, which is normally released in response to stress or reduced levels of serum glucocorticoids, regulates blood glucose levels by promoting gluconeogenesis, suppresses the immune system, and accelerates protein metabolism. Hypercortisolism is the hallmark of Cushing disease and causes a diverse array of symptoms, including central obesity, hypertension, hyperglycemia, osteoporosis, and skin and muscle atrophy.

Cushing disease is caused by an adenoma arising from pituitary corticotroph cells. These cells, which are found in the anterior or pituitary, produce ACTH from the precursor proopiomelanocortin (POMC) and then secrete it in response to corticotropin-releasing hormone from the hypothalamus (Figure 1). Treatment options for patients with Cushing disease are essentially limited to surgical resection. However, surgical resection alone has several important limitations (reviewed in ref. 1). First, preoperative studies to localize the tumor are not always definitive, since the majority of pituitary corticotroph adenomas are very small in size (less than 10 mm in diameter). Second, surgical remission rates for larger tumors are relatively low. Finally, postoperative recurrence rates range from 10% to 45%, depending on the size of the initial tumor.

Conflict of interest: The author has declared that no conflict of interest exists.

Citation for this article: J Clin Invest. 2011;121(12):4621–4623. doi:10.1172/JCI61127.