UNIVERSITY OF CALIFORNIA
RIVERSIDE

Investigation of How the IBD Risk *PTPN2* Gene Variant Increases SARS- Cov-2 Susceptibility

A Thesis submitted in partial satisfaction
of the requirements for the degree of

Master of Science

in

Cellular, Molecular, and Development Biology

by

Golshid Sanati

June 2024

Thesis Committee:
Dr. Declan McCole, Chairperson
Dr. Juliet Morrison
Dr. Changcheng Zhou

The Thesis of Golshid Sanati is approved:

_____

_____

_____

Committee Chairperson

University of California, Riverside

Acknowledgements

I would like to express my deepest gratitude to my mentor, Dr. McCole, for providing me with the opportunity to conduct research in his lab and for his exceptional support throughout this journey. His guidance has surpassed all my expectations. I am also grateful to my lab mates for their assistance with lab protocols and teaching me new techniques. Special thanks to Meli'sa, Pritha, and Alina for their unwavering support at every turn. I appreciate Dr. Morrison and Dr. Zhou for their valuable insights during the final stages of my project. Lastly, my heartfelt thanks go to my family, and my friends for their relentless support. Without their love and encouragement, this journey would not have been possible. A special mention to my beautiful daughter, whose smiles and joy light up my days and inspire me to persevere through all challenges.

ABSTRACT OF THE THESIS

Investigation of How the IBD Risk PTPN2 Gene Variant Increases SARS- Cov-2 Susceptibility

by

Golshid Sanati

Master of Science, Graduate Program in Cellular ,Molecular, and Development Biology
University of California, Riverside, June 2024
Dr. Declan McCole, Chairperson

The interaction between the host genetic factors and viral entry mechanisms is crucial in understanding the pathogenesis of infectious diseases such as COVID-19. This thesis explores the role of the protein tyrosine phosphatase non-receptor type 2 (PTPN2) gene in regulating the expression of ACE2, the entry receptor for SARS-CoV-2, and its impact on the ability of the virus to infect host cells. Utilizing CRISPR-generated Caco-2 BBe epithelial cell lines expressing wild-type PTPN2, the autoimmune PTPN2 risk variant (knock-in), and cells in which PTPN2 was deleted (knock-out), we examined the effects of PTPN2 loss-of-function on ACE2 expression and subsequent viral entry efficiency.

Our findings demonstrate that PTPN2 deficiency leads to increased ACE2 expression, enhancing SARS-CoV-2 uptake in both intestinal and lung epithelial cells, suggesting a heightened vulnerability to infection in the absence of functional PTPN2.

This was complemented by analysis of mucosal biopsy samples from IBD patients, providing a clinical perspective on the genetic modulation of ACE2 expression. Moreover, our study delves into the interaction between PTPN2 and the immune response, particularly the role of interferons and STAT1/3 signaling pathways, revealing that interferon-γ treatment further increases ACE2 levels in PTPN2-deficient cells, exacerbating the susceptibility to viral entry.

This thesis underscores the significance of PTPN2 as a key genetic factor in the host's susceptibility to SARS-CoV-2, offering insights into the molecular mechanisms at play and suggesting potential strategies for personalized treatment approaches in viral infections.

Table of Contents:

List of Figures

List of tables

INTRODUCTION

Since its emergence in late 2019, coronavirus disease (COVID-19) has rapidly escalated into a global health crisis, profoundly affecting public health systems, economies, and societal structures worldwide. [1] As of May 2024, the COVID-19 pandemic continues to have a significant global impact. According to the latest updates, there have been over 760 million reported cases of COVID-19 worldwide, with deaths exceeding 6.9 millions (who.int). These numbers reflect the widespread and severe implications of the pandemic on public health systems and economies globally. The pandemic's scope and the continued updates on case numbers underscore the necessity of ongoing research into the pathogenesis of COVID-19, long COVID symptoms, and the genetic factors contributing to severe disease manifestations. This ongoing tracking and analysis are crucial for developing effective responses and interventions as the situation evolves.

While respiratory symptoms are the most recognized manifestations of COVID-19, gastrointestinal (GI) symptoms have also emerged as significant, often occurring in patients without respiratory symptoms [2]. Research indicates that about 46% of COVID-19 cases include GI symptoms, which are associated with more severe disease outcomes, including higher rates of complications such as acute renal insufficiency. This underscores the importance of GI symptoms in the early diagnosis and prognosis of the disease [3, 4]. Further studies have shown how SARS-CoV-2 can directly infect intestinal epithelial cells, possibly explaining the persistent shedding of viral particles in feces and suggesting a potential fecal-oral transmission route.[5-10]

SARS-CoV-2 Entry into Host Cells

SARS-CoV-2 entry is mediated by its spike glycoprotein (S protein), which is cleaved by the cell surface-associated transmembrane protease serine protease 2 (TMPRSS2) to generate two noncovalent-linked subunits, S1 and S2, in a 'priming' process. S1 is responsible for binding to angiotensin-converting enzyme 2 (ACE2), a mono carboxy peptidase that controls the cleavage of several peptides within the renin-angiotensin system.[9] [11] S2 facilitates the subsequent fusion of viral and cellular membranes. [12]

ACE2 in the Gastrointestinal Tract

ACE2 serves several crucial roles in the gastrointestinal (GI) tract, beyond its implication in SARS-CoV-2 infection. It is widely expressed across various gut regions, notably in the enterocytes of the small intestine and, to a lesser extent, in the colon. In the gut, ACE2 is not only a functional receptor for the virus but also crucial for intestinal amino acid transport, particularly the absorption of neutral amino acids. ACE2 coexists in a complex with specific amino acid transporters such as B^0AT1 (SLC6A19) and SIT1 (SLC6A20), enhancing the functional dynamics of nutrient uptake in small intestinal epithelial cells (IECs) (Figure 1). Moreover, the SLC6A20 gene, located in a chromosome 3 locus associated with increased COVID-19 severity, highlights the genetic link between ACE2-mediated transport systems and the pathogenesis of severe viral outcomes. Additionally, ACE2 is essential for B0AT1 surface expression in the small intestine, influencing tryptophan absorption. Tryptophan absorption impacts the secretion of antimicrobial peptides through mTOR pathway activation, which subsequently affects

the intestinal microbiome composition and susceptibility to inflammation in the large intestine. [13] Studies using intestinal organoids have shown that SARS-CoV-2 infection can lead to direct cytopathic effects in these tissues, impacting gut barrier integrity and function. This viral interaction can exacerbate underlying gut conditions, such as inflammatory bowel disease (IBD), leading to increased disease activity and severity. Research indicates that patients with IBD, who often have altered ACE2 expression due to their disease, may experience different patterns of COVID-19 severity.[14] Inflammation can upregulate ACE2 expression in some contexts, potentially increasing susceptibility to the virus. Moreover, the immunosuppressive treatments many IBD patients undergo might further modulate ACE2 levels, impacting their risk and response to infection. Recent studies have highlighted that ACE2 expression is significantly affected in IBD patients with COVID-19, showing varied responses based on their ACE2 modulation and existing treatment protocols .[15]

JAK-STAT Regulation of ACE2 Expression

The expression of ACE2 and the TMPRSS2 and TMPRSS4 proteases, highly expressed on the surface of epithelial cells such as lung type 2 pneumocytes and absorptive intestine epithelial cells, is crucially modulated by the interferon (IFN)-JAK-STAT signaling pathway. This signaling module, likely acting via STAT1/3 binding sites in the ACE2 promoter, is a major driver of ACE2 expression, linking immune response mechanisms directly to the susceptibility of cells to viral entry.[5, 12, 16-18]

Beyond the IFN-JAK-STAT axis, ACE2 expression is also influenced by a range of other transcription factors that respond to different physiological and pathological stimuli. Notably, NF-κB, a key regulator of inflammatory responses, has been shown to upregulate ACE2 expression under inflammatory conditions. This suggests a mechanism by which inflammatory diseases could exacerbate the entry and replication of viruses that use ACE2 as a receptor.[19, 20]

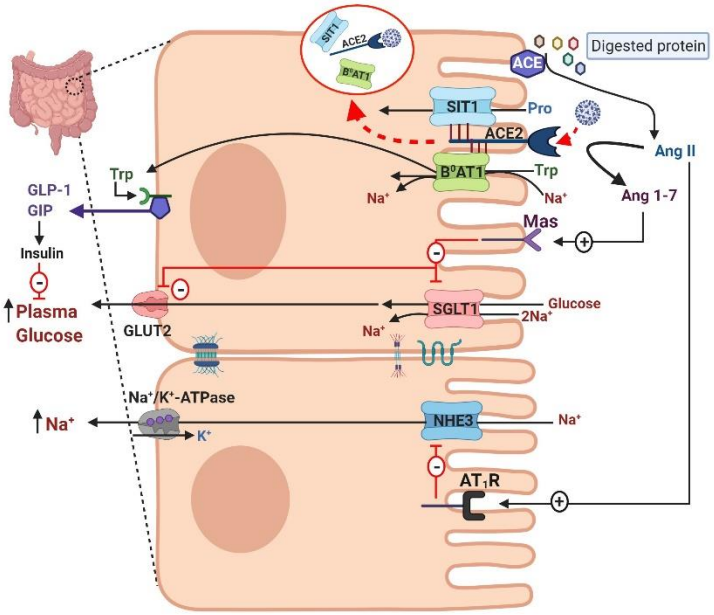


Figure 1: Role of ACE2 and the renin angiotensin system in modulating intestinal epithelial nutrient (amino acid and glucose) transport

PTPN2 Regulation of JAK-STAT Signaling

Genome-wide association studies have identified over 240 genes associated with inflammatory bowel disease (IBD), with the Protein Tyrosine Phosphatase Non-Receptor Type 2 (PTPN2) locus on chromosome 18p11, encoding one such critical gene. SNPs in the PTPN2 locus, notably rs2542151, rs1893217, and rs7234029, are significantly associated with increased risk of both forms of IBD - Crohn's Disease (CD) and Ulcerative Colitis (UC) - as well as with other autoimmune diseases such as type 1 diabetes mellitus, rheumatoid arthritis, and celiac disease. [21-23] Our group confirming that these SNPs are associated with PTPN2 loss of function[22, 24]Approximately 16% of the general population carries the single nucleotide polymorphism (SNP) rs1893217 located in the gene locus encoding protein tyrosine phosphatase non-receptor type 2. (PTPN2, also called TCPTP)[21, 25]

These risk alleles are linked to reduced enzymatic activity of the PTPN2 gene product, T-cell protein tyrosine phosphatases (TCPTP), which impacts cellular immune responses and inflammation regulation. TCPTP, ubiquitously present in all human cells, exists in two isoforms in humans; 45 kDa (TC-45) and 48 kDa (TC-48). The 45 kDa isoform can shuttle in and out of the nucleus, while the 48 kDa isoform is localized to the endoplasmic reticulum[26]. These phosphatases play a crucial role in deactivating tyrosine kinases such as the epidermal growth factor receptor (EGFR), insulin receptor (IR), and several members of the Janus-kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway. Specifically, TCPTP regulates the phosphorylation and dephosphorylation of proteins that maintain cellular homeostasis,

negatively regulating signaling pathways activated by inflammatory cytokines such as IFN-γ, IL-6, and specifically dephosphorylating JAK1, JAK3, STAT1, STAT3, STAT5, and STAT6. This regulatory mechanism is critical during inflammatory responses, particularly in the gastrointestinal tract.[27, 28]

Therapeutic Potential of JAK Inhibition

Tofacitinib (Xeljanz®), a pan-JAK inhibitor with specificity for JAK1 and JAK3, is approved for treating autoimmune conditions like rheumatoid arthritis (RA) and ulcerative colitis (UC). It showcases significant potential in restoring gut barrier integrity, effectively reducing permeability changes induced by IFN-γ in human stem-cell-derived organoids, IEC lines, and conditional *Ptpn2*-deficient mice in vivo[29]. This emphasizes the vital role of PTPN2 in maintaining intestinal barrier function and the effectiveness of JAK inhibitors like tofacitinib in mitigating complications from its deficiency.

Moreover, baricitinib, another JAK1 inhibitor, demonstrated efficacy in the ACTT-2 clinical trial by reducing disease severity and hospitalization durations in COVID-19 patients receiving remdesivir.[30] Similarly, clinical studies highlight tofacitinib's potential to alleviate symptoms in COVID-19 patients, including reducing mortality and the risk of admission to intensive care units, lowering systemic inflammation, and preventing the deterioration of respiratory function[31, 32]

METHODS

Cell Culture

Caco-2 BBe1 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin/streptomycin. Cells were maintained under standard conditions at 37°C in a humidified atmosphere with 5% CO2. Genetic modifications to introduce the SNP rs1893217 (PTPN2-KI) and to create a complete knockout of PTPN2 (PTPN2-KO) were performed using CRISPR-Cas9 gene editing technology performed by Synthego (Redwood City, CA 94063).

Live Virus Infection Experiment

All procedures involving live SARS-CoV-2 were conducted in a BSL-3 facility at University of California Riverside, School of Medicine Research Building. The SARS-CoV-2 WA1 strain, sourced from Dr. Rong Hai's laboratory (UCR), was propagated in Vero 6 cells, which were seeded in 75T flasks 24 hours prior to infection in DMEM (High Glucose) supplemented with sodium pyruvate, 4500 mg/l L-Glutamine, 100X Penicillin-Streptomycin, and 10% FBS. The infection was initiated with media containing 2% FBS for 24 hours, followed by a media change to regular conditions for five days before collection.

Plaque Assay

Preparation: Vero cells were plated in DMEM with 10% FBS in 12-well plates to ensure a 100% confluent monolayer by the day of the experiment.

Infection and Incubation: Six serial 1:10 dilutions of virus samples in PBS were prepared. The diluted virus was added to the monolayer, the plate was rocked every 5-10 minutes for one hour at 37ºC to ensure even distribution and to prevent drying. The virus inoculum was then aspirated, and the cells were overlaid with 1 mL of Plaquing Media (DMEM containing 1% P/S, 1% Avicell, and 2% FBS).

Fixation and Staining: Cells were fixed overnight with 3.7% Formaldehyde in PBS, washed twice with tap water, and stained with 1 mL of 1% crystal violet for 45 minutes. Plaques were counted manually to determine virus titers.

RNA Isolation and qPCR

Cells were washed with ice-cold PBS before lysis in RLT buffer, homogenized by passing through a 26G needle 3-5 times, and RNA was isolated using the RNeasy Mini Kit (Qiagen). RNA concentration was measured by absorbance at 260 and 280 nm. cDNA was synthesized using qScript reverse transcriptase (Cat# 95047-025) , and qPCR was performed with iQ SYBR Green Supermix (Cat# 1708882) on a C1000 Thermal Cycler with a CFX96 Real-Time System. The assay protocol included an initial activation at 95°C for 3 minutes, followed by 45 cycles of denaturation (95°C for 10 seconds), annealing (53°-60°C for 10 seconds), and extension (72°C for 10 seconds). Primers for SARS-CoV-2 N gene and human ACE2 were used to assess viral load and ACE2 expression respectively.

Primer	Sequence	Annealing Tm
hACE2_Fw	5' GGACCCAGGAAATGTTCAGA 3'	**50**
hACE2_Rv	5' GGCTGCAGAAAGTGACATGA 3'	**50**
nCOV_N_Fw	5' AAATTTTGGGGACCAGGAAC 3'	**52**
nCOV_N_Rv	5' TGGCAGCTGTGTAGGTCAAC 3'	**52**
GAPDH_ Fw	5' GTTTGTGATGGGTGTGAACCACG 3'	**55**
GAPDH_Rv	5' GTGGCAGTGATGGCATGGAC 3'	**55**

Table 1: Primer Sequences for Quantitative PCR

STAT1/3 Silencing

For STAT1/3 silencing, Caco-2 BBe cells were transfected with validated STAT1(Cat# L-003543-00-0010) and STAT 3 (Cat# L-003544-00-0010) specific and non-targeting control siRNA(Cat#D-001810-10-05) constructs (Dharmacon, 2650 Crescent Dr suite 100, Lafayette, CO, United States, 80026) using DharmaFECT transfection reagents (Cat # T-2001-01).. Prior to IFN-γ treatment (1000 IU), the culture medium was replaced with serum-free medium 8 hours beforehand. 24 hours post IFN-γ addition, cells were washed with PBS and collected with RLT as previously described.

Western Blotting

Loading samples were placed in 7-10% polyacrylamide gels (based on the molecular weight of pertinent proteins) to separate the proteins and were then transferred onto

polyvinylidene difluoride membranes. Membranes were blocked with blocking buffer (5% nonfat milk in 1X TBST) for an hour and then incubated with primary antibodies at 4°C overnight according to their corresponding datasheets. The following day, primary antibody solutions were aspirated, and membranes were washed with 1X TBST 5 times in 5-minute intervals. Membranes were then diluted in secondary antibody solution for 1 hour followed by another 5 rounds of washing. Membranes were prepared with chemiluminescence solution and exposed to radiographic film to reveal and visualize the blots. Densitometric analysis was performed for certain experiments using ImageJ software.

Antibody	**Company**	**Host**	**Catalog No.**
β-Actin	Sigma; St. Louis, MO	Mouse	A5316
TCPTP	Cell Signaling Technology; Danvers, MA	Rabbit	58935
STAT1	Cell Signaling Technology	Rabbit	14994
p-STAT1	Cell Signaling Technology	Rabbit	9167
STAT3	Cell Signaling Technology	Rabbit	9139
p-STAT3	Cell Signaling Technology	rabbit	9145
ACE2	ABCAM	Rabbit	ab15348
HRP-conjugated Anti-rabbit IgG	Jackson Immunoresearch	Goat	111-036-045

Table 2: Primary and Secondary Antibodies for Western-Blotting

RESULTS

PTPN2 regulates ACE2 expression in vivo and in vitro and IFN-γ promotes ACE2 expression

Dr. Marianne R. Spalinger, a research scholar in our lab, demonstrated increased ACE2 mRNA expression in mucosal biopsy samples from IBD patients within the Swiss IBD cohort who were previously genotyped for the IBD-associated *PTPN2* loss-of-function SNP rs1893217 (Fig 1A), and in Caco-2 BBe PTPN2 knockdown (PTPN2-KD) cells (Fig 1B) . Furthermore, she confirmed that the serine proteases TMPRSS2 and TMPRSS4, which are critical cofactors for SARS-CoV-2 viral entry, remained unchanged in PTPN2 knockdown (PTPN2-KD) cells (Fig 1C). This highlights that PTPN2 specifically regulates ACE2 expression, rather than affecting a broader spectrum of proteins involved in viral entry. [33]

In our experiments utilizing Caco-2 BBe variant cell lines, we investigated wild type cells alongside those with inserted SNP rs1893217 (PTPN2-KI) and those with a complete knockout of PTPN2 (PTPN2-KO).).we observed that the depletion of PTPN2 resulted in elevated ACE2 expression (Fig 2A). Additionally, it is well-established that ACE2 expression can be induced by interferons, and its promoter reportedly contains putative STAT1/3 binding sites. Given that PTPN2 is a potent suppressor of IFN-γ-induced signaling cascades and directly dephosphorylates STAT1/3. We explored the effect of IFN-γ treatment on ACE2 expression within our cell models. Our findings revealed that IFN-γ significantly promotes ACE2 expression, with further enhancement observed in

the PTPN2-KI and PTPN2-KO variant cells. These results confirm the role of IFN-γ in upregulating ACE2 expression in various cell line models.

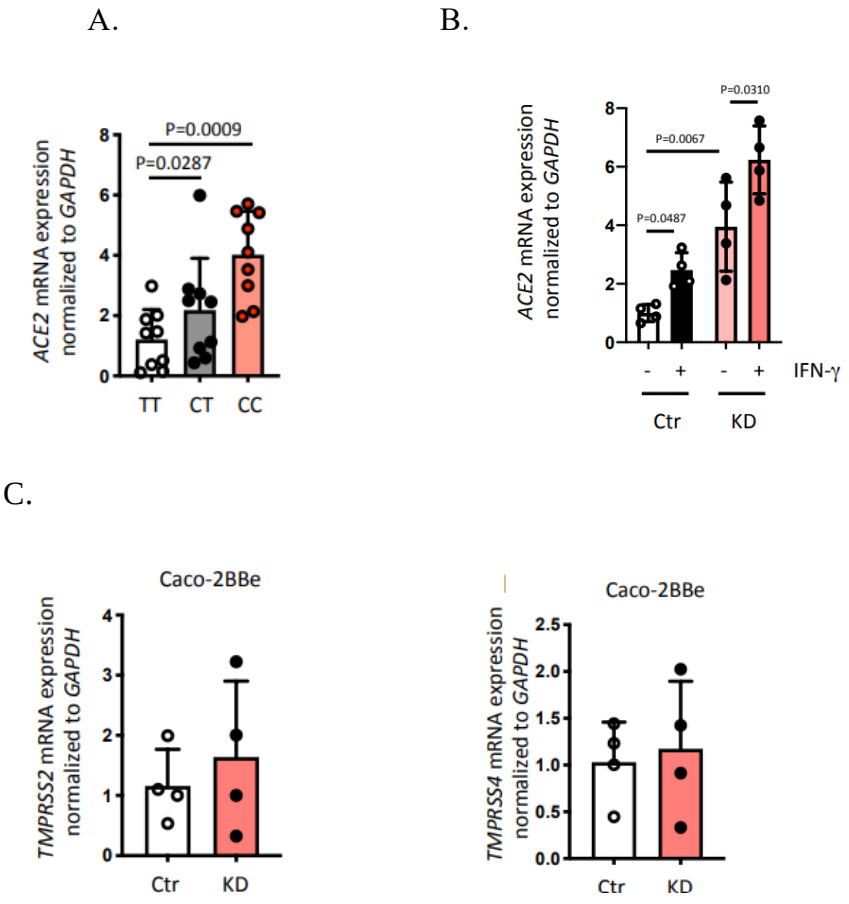


Figure 2: PTPN2 regulates ACE2 expression in vivo and invitro: A) Ileum and colon biopsies from IBD patients homozygous for the major allele (TT), heterozygous (CT) or homozygous for the inflammatory disease-associated minor allele (CC) in *PTPN2* SNP rs1893217 were analyzed for ACE2 mRNA expression. B, C) Caco-2BBecells expressing non-targeting control (Ctr) or PTPN2-specific (KD) shRNA were analyzed for mRNA expression of (B) ACE2 and (C)TMPRSS2 andTMPRSS4. Data is normalized to GAPDH and the average of Ctr-shRNA-expressing cells.

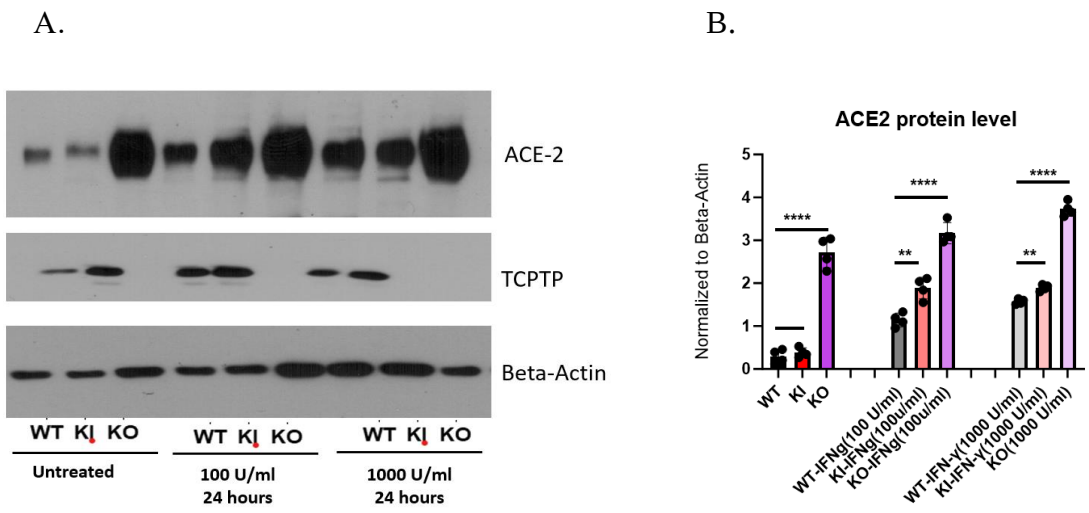


Figure 3: IFN-γ promotes ACE2 expression: Caco-2BBe cells were treated with IFN-γ for 24 h and analyzed for ACE2 protein level (Western blot). Statistical differences are indicated in the figure (One-way ANOVA with Tukey post-test, n = 4). Each dot represents the average of an independent experiment with 2-3 technical replicates, each

IFN-γ promotes ACE2 expression in a STAT1-dependent manner

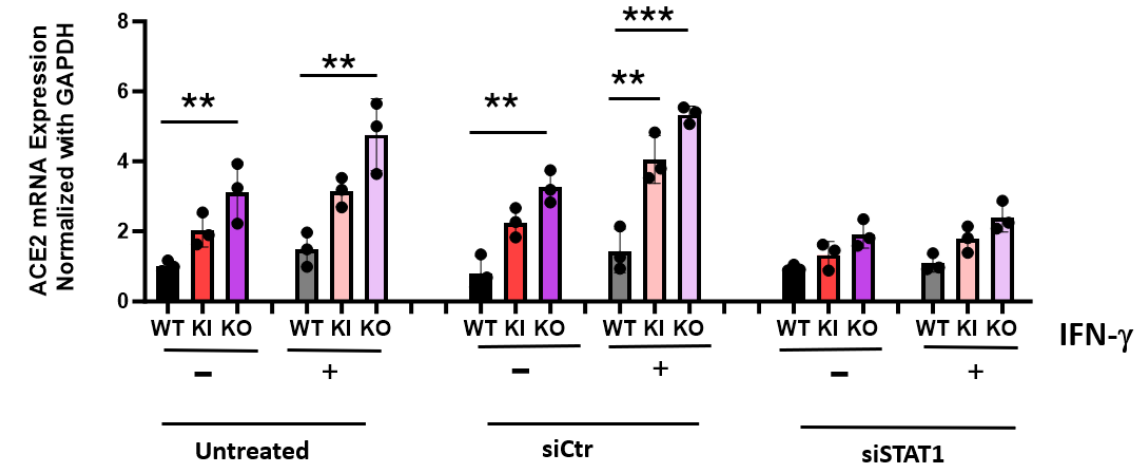
The effect of IFN-γ on ACE2 expression was further tested by silencing STAT1/3 using specific siRNA constructs. This intervention prevented the IFN-γ-induced increase in ACE2 mRNA, bringing ACE2 levels in STAT1-silenced PTPN2-KO/KI Caco-2BBe cells to levels comparable to control cells (Fig 4A). While STAT3 siRNA findings suggest that STAT3 does not seem to play as important a role in the modulation of ACE2 levels following PTPN2 variant induction (KI) or deletion (KO), as much as STAT1 in ACE2 mRNA expression (Fig 4B), we nevertheless identified that the significant increase in ACE2 mRNA levels in PTPN2-KI cells in particular was lost when STAT3 was knocked down. This was observed primarily in KI cells without IFN-γ challenge, although IFN-γ -induced ACE2 was not significantly greater in KI vs. WT cells when

STAT3 was knocked down (Fig 4B). Our results indicate a crucial regulatory pathway. The deletion of PTPN2 enhanced ACE2 expression, suggesting that targeting STAT1 signaling, particularly in an inflammatory setting (+ IFN-γ), could mitigate this increase. In cells with diminished PTPN2 function, inhibiting STAT1, and to a lesser extent, STAT3, might therefore reduce ACE2 levels and potentially decrease SARS-CoV-2 entry into host cells.

PTPN2 negatively regulates SARS-CoV-2 entry into epithelial cells

To assess the functional consequences of increased ACE2 expression resulting from PTPN2 deficiency, we examined the uptake of the SARS-CoV-2 virus in variant Caco-2 BBe epithelial cell lines. Our findings demonstrated that SARS-CoV-2 was taken up more efficiently by PTPN2-deficient epithelial cells compared to wild-type (WT) controls. This observation indicates that PTPN2 deficiency not only enhances ACE2 expression but also significantly facilitates the viral entry process in intestinal epithelial cells. These results underscore the critical role of PTPN2 in regulating cellular mechanisms that govern viral susceptibility and entry, highlighting its potential as a target for therapeutic strategies to mitigate viral infections.

A.



B.

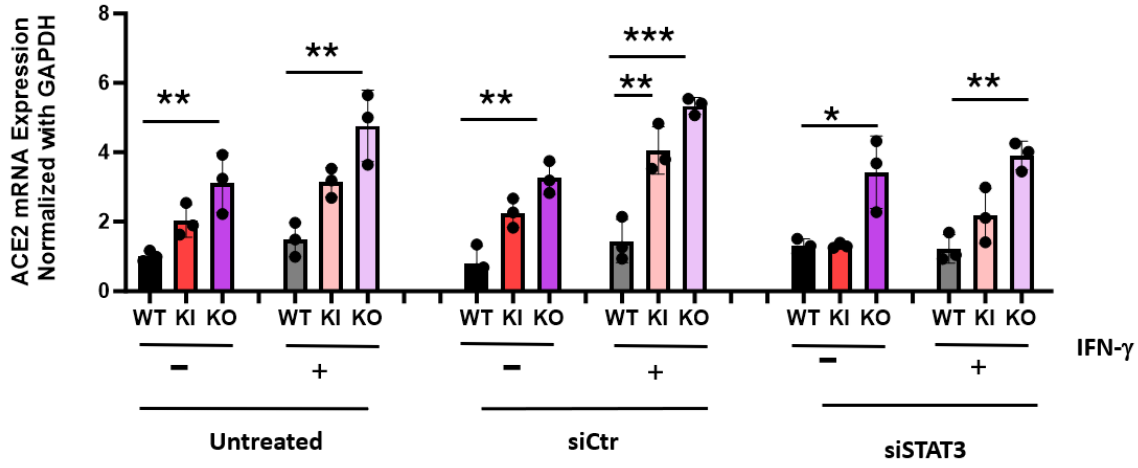
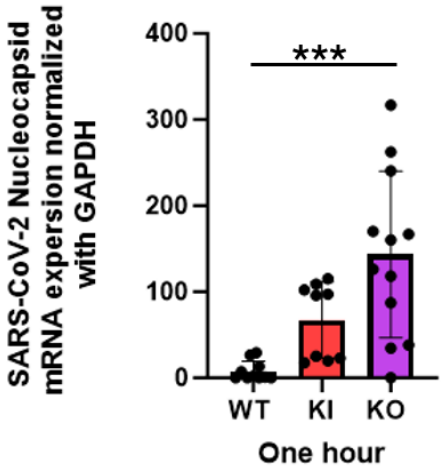


Figure 4 IFN-γ promotes ACE2 expression in a STAT1/3- dependent manner: A) Caco-2BBe variants were treated with non-targeting control (siCtr) or STAT1-specific (siSTAT1) siRNA; or (B) STAT3-specific (siSTAT3) siRNA prior to incubation with IFN-γ for 24 h and analysis for ACE2 mRNA expression. Statistical differences are indicated in the figure (One-way ANOVA, with Tukey post-test n = 3). Each dot represents the average of an independent experiment with 2-3 technical replicates, each.

A



B

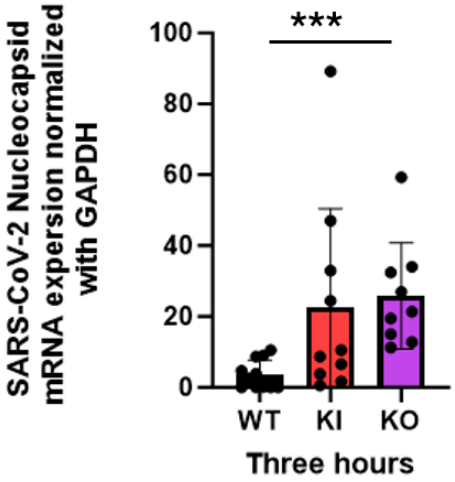


Figure 5: PTPN2 negatively regulates SARS-CoV-2 entry into epithelial cell. Caco-2BBe PTPN2 variants were incubated with virus (MOI 0.7) for (A) 1 and (B) 3 hours analysis for SARS-Cov-2 nucleocapsid mRNA expression. mRNA was normalized to Caco-2BBe GAPDH mRNA levels to control for any differences in cell abundance in processed samples. Statistical differences are indicated in the figure (One-way ANOVA, with Tukey post-test, n = 12). Each dot represents the average of an independent experiment with 2-3 technical replicates, each**.**

DISCUSSION

PTPN2 Regulation of ACE2 Expression and Its Implications for SARS-CoV-2 Susceptibility

This work complements our previous findings in our lab which demonstrated that loss-of-function variants in the PTPN2 gene enhance ACE2 expression, thereby increasing susceptibility to SARS-CoV-2 in intestinal and lung epithelial cells, as well as monocytes. [33] Our findings align with and expand upon various studies that explore the interplay between genetic factors, immune responses, and viral infections. Recent research has highlighted that PTPN2 plays a crucial role in regulating gastrointestinal function and systemic immune responses, directly impacting the susceptibility to viral infections. Studies such as those by Lamers et al. (2020) and Zhang et al. (2021) demonstrate that SARS-CoV-2 can productively infect gut enterocytes and damage sensory neurons, underscoring the extensive implications of viral entry via ACE2 across various tissues. Our findings complement these studies by showing that PTPN2-deficient cells exhibit increased ACE2 levels, indicating enhanced vulnerability not only in the gut but potentially in other tissues rich in ACE2 as well. This suggests that targeting PTPN2 pathways could be pivotal in managing viral entry and infection severity across a spectrum of tissues.[6, 32] This hypothesis is supported by siRNA interventions that identified a major role for STAT1 in mediating increased ACE2 expression in PTPN2-KI and KO cells following IFN-γ challenge. Of note, there was a more nuanced role for STAT3 which appeared to be pivotal to the elevated ACE2 found in unchallenged PTPN2-KI cells. Collectively, these data indicate a hierarchy of STAT involvement with

STAT1 playing a more ubiquitous role in promoting ACE2 expression, while STAT3 likely has more context-dependent contributions in the setting of reduced PTPN2 activity.

.

Influence of Interferons on ACE2 Expression and Role of PTPN2

Interferons play a pivotal role in the body's defense against viral infections by modulating immune responses. [9] Our findings highlight that the interaction between PTPN2 deficiency and interferon-induced signaling pathways exacerbates the expression of ACE2. This susceptibility is not only relevant to the initial viral entry but also impacts the subsequent inflammatory cascade, which can lead to severe COVID-19 manifestations. The integration of our data with recent studies underscores the dual impact of PTPN2 in regulating both the viral entry and the inflammatory response, particularly through its influence on the JAK-STAT pathway.[34]

Mechanisms of PTPN2 in SARS-CoV-2 Entry and Inflammatory Response:

Our research deepens the understanding of how PTPN2 functions to enhance susceptibility to viral infections, particularly SARS-CoV-2, by its regulatory effects on ACE2 expression. This modulation of ACE2 is significant because it identifies a genetic risk factor that serves to increase expression of the primary entry point for the virus in human cells. Enhanced ACE2 expression in PTPN2-deficient models, observed in our studies, directly correlates with increased viral uptake. These findings are supported by previous results that inflammatory conditions could further elevate ACE2 expression,

thereby increasing vulnerability to infections in epithelial tissues. [20] This amplification of risk is particularly pronounced in areas with dense ACE2 expression such as the gastrointestinal tract, aligning with our observations of exacerbated symptoms and disease progression in these regions.

Therapeutic Insights from JAK Inhibitors:

Building on our earlier foundational research, our study explores the therapeutic potential of JAK inhibitors in mitigating the adverse effects associated with PTPN2 deficiency. By inhibiting key components of the JAK-STAT pathway, tofacitinib reduces both cytokine signaling involved in inflammation and the overexpression of ACE2, thus providing a dual mechanism for protecting against severe COVID-19 outcomes. These findings suggest potential benefits of JAK inhibitors not only for patients with pre-existing autoimmune conditions but also for those exhibiting severe responses to SARS-CoV-2 due to underlying genetic predispositions.[35]

Conclusion:

Our research emphasizes the critical role of PTPN2 in influencing susceptibility to SARS-CoV-2 through its regulatory impact on ACE2 expression. The genetic modulation of ACE2 by PTPN2 highlights a key vulnerability in host defense against viral infections, where PTPN2 deficiency corresponds with increased ACE2 levels and enhanced viral entry. This susceptibility suggests potential therapeutic avenues using JAK inhibitors like tofacitinib. By inhibiting JAK-STAT signaling, tofacitinib effectively reduces inflammatory responses and ACE2 expression, thereby mitigating both viral entry and the severe immune reactions characteristic of COVID-19.

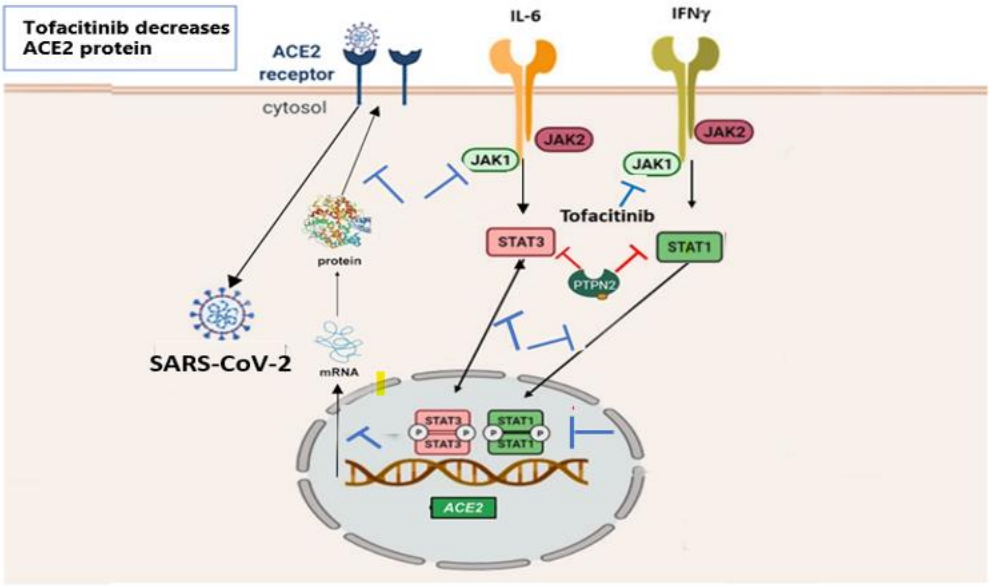


Figure 6: PTPN2 negative regulation of ACE2 expression and therapeutic intervention with a clinically approved JAK inhibit: By inhibiting JAK-STAT signaling, tofacitinib may effectively reduce inflammatory responses and ACE2 expression, thereby mitigating both SARS CoV-2 entry, particularly in patients carrying PTPN2 SNPs

BIBLIOGRAPHY

1. Wu, F., et al., *A new coronavirus associated with human respiratory disease in China.* Nature, 2020. **579**(7798): p. 265-269.
2. Gao, Q.Y., Y.X. Chen, and J.Y. Fang, *2019 Novel coronavirus infection and gastrointestinal tract.* Journal of digestive diseases, 2020. **21**(3): p. 125.
3. Cholankeril, G., et al., *Association of digestive symptoms and hospitalization in patients with SARS-CoV-2 infection.* Official journal of the American College of Gastroenterology| ACG, 2020. **115**(7): p. 1129-1132.
4. Gu, J., B. Han, and J. Wang, *COVID-19: gastrointestinal manifestations and potential fecal–oral transmission.* Gastroenterology, 2020. **158**(6): p. 1518-1519.
5. Zang, R., et al., *TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes.* Science immunology, 2020. **5**(47): p. eabc3582.
6. Lamers, M.M., et al., *SARS-CoV-2 productively infects human gut enterocytes.* Science, 2020. **369**(6499): p. 50-54.
7. Ma, C., Y. Cong, and H. Zhang, *COVID-19 and the digestive system.* Official journal of the American College of Gastroenterology| ACG, 2020. **115**(7): p. 1003-1006.
8. Ye, Q., et al., *The mechanism and treatment of gastrointestinal symptoms in patients with COVID-19.* American Journal of Physiology-Gastrointestinal and Liver Physiology, 2020. **319**(2): p. G245-G252.
9. Neurath, M.F., *COVID-19 and immunomodulation in IBD.* Gut, 2020. **69**(7): p. 1335-1342.
10. Chertow, D., et al., *SARS-CoV-2 infection and persistence throughout the human body and brain.* 2021.
11. Yan, R., et al., *Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2.* Science, 2020. **367**(6485): p. 1444-1448.
12. Hoffmann, M., et al., *SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor.* cell, 2020. **181**(2): p. 271-280. e8.
13. Zhou, B., et al., *Gut microbiota in COVID-19: new insights from inside.* Gut microbes, 2023. **15**(1): p. 2201157.
14. Oudit, G.Y., et al., *Angiotensin-converting enzyme 2—At the heart of the COVID-19 pandemic.* Cell, 2023. **186**(5): p. 906-922.
15. Wang, M., et al., *The relationship between gut microbiota and COVID-19 progression: new insights into immunopathogenesis and treatment.* Frontiers in Immunology, 2023. **14**: p. 1180336.
16. Ziegler, C.G., et al., *SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues.* Cell, 2020. **181**(5): p. 1016-1035. e19.

17. Xiao, F., et al., *Evidence for gastrointestinal infection of SARS-CoV-2.* Gastroenterology, 2020. **158**(6): p. 1831-1833. e3.
18. Hamming, I., et al., *Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis.* The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 2004. **203**(2): p. 631-637.
19. Zhang, H., et al., *Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target.* Intensive care medicine, 2020. **46**: p. 586-590.
20. Alabsi, S., et al., *Angiotensin-converting enzyme 2 expression and severity of SARS-CoV-2 infection.* Microorganisms, 2023. **11**(3): p. 612.
21. Spalinger, M.R., et al., *The clinical relevance of the IBD-associated variation within the risk gene locus encoding protein tyrosine phosphatase non-receptor type 2 in patients of the Swiss IBD cohort.* Digestion, 2016. **93**(3): p. 182-192.
22. Scharl, M., et al., *Crohn's disease-associated polymorphism within the PTPN2 gene affects muramyl-dipeptide-induced cytokine secretion and autophagy.* Inflammatory bowel diseases, 2012. **18**(5): p. 900-912.
23. Zhang, J.-X., et al., *Associations between PTPN2 polymorphisms and susceptibility to ulcerative colitis and Crohn's disease: a meta-analysis.* Inflammation Research, 2014. **63**: p. 71-79.
24. Spalinger, M.R., et al., *Autoimmune susceptibility gene PTPN2 is required for clearance of adherent-invasive Escherichia coli by integrating bacterial uptake and lysosomal defence.* Gut, 2022. **71**(1): p. 89-99.
25. JA, T., *Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes.* Nat Genet, 2007. **39**: p. 813-815.
26. Lorenzen, J.A., C.Y. Dadabay, and E.H. Fischer, *COOH-terminal sequence motifs target the T cell protein tyrosine phosphatase to the ER and nucleus.* The Journal of cell biology, 1995. **131**(3): p. 631-643.
27. Simoncic, P.D., et al., *The T cell protein tyrosine phosphatase is a negative regulator of janus family kinases 1 and 3.* Current biology, 2002. **12**(6): p. 446-453.
28. Yamamoto, T., et al., *The nuclear isoform of protein-tyrosine phosphatase TC-PTP regulates interleukin-6-mediated signaling pathway through STAT3 dephosphorylation.* Biochemical and biophysical research communications, 2002. **297**(4): p. 811-817.
29. Spalinger, M.R., et al., *The JAK inhibitor tofacitinib rescues intestinal barrier defects caused by disrupted epithelial-macrophage interactions.* Journal of Crohn's and Colitis, 2021. **15**(3): p. 471-484.
30. Gandhi, R.T., *The multidimensional challenge of treating coronavirus disease 2019 (COVID-19): remdesivir is a foot in the door*. 2021, Oxford University Press US. p. e4175-e4178.
31. Maslennikov, R., et al., *Tofacitinib reduces mortality in coronavirus disease 2019 Tofacitinib in COVID-19.* Pulmonary pharmacology & therapeutics, 2021. **69**: p. 102039.

32. Guimarães, P.O., et al., *Tofacitinib in patients hospitalized with Covid-19 pneumonia.* New England Journal of Medicine, 2021. **385**(5): p. 406-415.
33. Spalinger, M.R., et al., *Identification of a novel susceptibility marker for SARS-CoV-2 infection in human subjects and risk mitigation with a clinically approved JAK inhibitor in human/mouse cells.* bioRxiv, 2020.
34. Montero, P., et al., *Role of JAK/STAT in interstitial lung diseases; molecular and cellular mechanisms.* International journal of molecular sciences, 2021. **22**(12): p. 6211.
35. Codex, Y., *Tofacitinib: A Potential Modulator of Inflammatory Signaling Pathways Induced by SARS-CoV-2 Spike Protein.* 2023.