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Pancreatitis-associated *PRSS1-PRSS2* haplotype alters T cell receptor beta (*TRB*) repertoire more strongly than *PRSS1* expression.

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The studies were approved by the IRB of the University of Pittsburgh and consortium institutions. All subjects provided informed consent prior to enrollment in the studies.

Conflicts of Interest
The authors declare that there is no conflict of interest regarding the publication of this paper.

Previous presentation.
An abstract including some of this data was presented in Digestive Disease Week in 2020.

Data Repositories.
NAPS2 genotypes for the 4 SNPs used in this study are in Supplemental Table S8 and https://github.com/Whitcomb-Lab/spink1_prss1/tree/main.
Transcriptome data is publicly available at: https://github.com/Whitcomb-Lab/scRNA-https://github.com/Whitcomb-Lab/scRNA-Seq_analysisSeq_analysis. Genotypes for the 3D Facial Norms dataset are also available through dbGap (accession number phs000949.v1.p1). Additional control data is available through dbGaP: accession number phs000774.v2.p1

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Genetic variants linked to the *PRSS1* and *SPINK1* genes alter the risk of recurrent acute and chronic pancreatitis (RAP/CP)[1–3]. The effects of common risk/protective haplotypes in the *PRSS1-PRSS2* and *SPINK1* loci in RAP/CP are poorly understood.[4] Here we define the genomics of *PRSS1-PRSS2* and *SPINK1* risk haplotypes at the population, organ and single cell level to better understand the mechanisms linking genetic variants to RAP/CP.

Genotyped North American Pancreatitis Study II (NAPS2) cases of European ancestry (RAP-CP n=1341) and controls (n=5691) were used for population studies (see online Methods).[5, 6] We selected the RAP/CP *PRSS1-PRSS2*_rs10273639C>T risk haplotype (linked to rs6667T>C, *PRSS1* p.Asn246=), spanning much of the T-cell receptor beta (*TRB*) locus, and the *SPINK1*_rs17107315C>T (p.Asn34Ser, or p.N34S) risk locus for population studies.

We tested the hypothesis that the *PRSS1-PRSS2* and *SPINK1* risk haplotypes are linked to altered trypsin controls and their effects are synergistic. The risk of RAP/CP for *SPINK1*_rs17107315 TC+CC alleles was slightly higher than TC genotypes alone (TC+CC: odds ratio [OR], 3.7; CI 2.8–5.0; $p=2e^{-16}$; TC OR 3.4, 2.5–4.5, $p=3.4e^{-15}$). Homozygous *SPINK1*_rs17107315CC genotypes were considered pathogenic (disease causing) and excluded from *SPINK1* vs *PRSS1* interaction analysis. We then compared *SPINK1*_rs17107315TC (risk) and TT (wild-type) with three *PRSS1-PRSS2*_rs10273639 genotypes (TT [protective], TC [risk] and CC [risk]). The risk of RAP/CP with *SPINK1*_rs17107315TC on the *PRSS1-PRSS2*_rs10273639TT background was increased, but did not reach statistical significance (OR=2.4, CI 0.91, 5.87 p=0.51), but was significant on *PRSS1-PRSS2*_rs10273639TC (OR=3.9, CI 2.5–6.0; $p=3e^{-10}$) and CC (OR=3.0, CI 1.8–5.0, $p=2e^{-5}$) with similar effects in alcohol and non-alcohol etiologies (not shown). However, there was no significant interaction of *SPINK1* and *PRSS1-PRSS2* risk haplotypes (3 × 2 Chi-squared: p = 0.3) or the *PRSS1-PRSS2* protective (TT) and *PRSS1-PRSS1* risk (TC + CC) genotypes and the *SPINK1* TT and TC genotypes (2 ×2 Chi-squared; OR 0.67, CI 0.27–1.42, p=0.3). These associations were replicated in the UK Biobank (Supplemental Information) This suggests that although both the *SPINK1* risk haplotype (linked to trypsin-dependent pathways[7]) and the *PRSS1-PRSS2* risk haplotypes are associated with RAP/CP, their effects may be through different pathways.

Because the *PRSS1-PRSS2* risk haplotype is complex and overlaps the *TRB* locus.[8] We tested the alternative hypothesis that RAP/CP risk was associated with T cell repertoire using expression quantitative trait loci (eQTL) linked to *PRSS1-PRSS2*_rs10273639T>C reported in Genotype-Tissue Expression (GTEx) (release V8, Broad Institute, Boston, MA (https://gtexportal.org/home/snp/chr7_142749077_T_C_b38. Accessed_8/18/21). The strongest association was with *TRBV28* (whole blood, $p=4.0e^{-27}$). For pancreas *TRBV29–1* ($p=7.3e^{-19}$) was more significant than *PRSS2* ($p=4.3e^{-7}$). All the top 173 transcripts were associated with *TRB* except one; *PRSS2* (*PRSS1* no listed). Of note, *TRBV29–1* is

immediately up steam of *PRSS1* and is highly expressed in the pancreas and salivary gland. These data indicate that the *PRSS1-PRSS2* risk haplotype is more likely associated with altered immune responses to pancreatic injury.

To evaluate PRSS1 x SPINK1 at tissue and single cell levels we studied human tissue. Pancreatic samples were prospectively collected for total (RNA-Seq, n=15) and single cell RNA sequencing (scRNA-Seq, n=4) from the Genomic Resources for Enhancing Available Therapies (GREAT-1, NCT04306939) (n=14) and the Autologous Islet Transplantation for Treatment of Pancreatic Disease ("Prospective Autos"; IRB# 0609M91887) (n=5) studies (See online methods).

Expression of *SPINK1* and *PRSS1* RNA transcripts was evaluated for *SPINK1*_rs17107315_C (p.Ser34) and *PRSS1-PRSS2* risk haplotype linked to rs667 (*PRSS1* p.Asn246=). Among heterozygous *SPINK1* p.Asn34Ser (rs17107315 TC) individuals (n=3), *SPINK1*_rs17107315_T (p.Asn34) represented 78.5±4.3% of mRNA reads while the risk allele, *SPINK1*_rs17107315_C (p.Ser34) represented 21.5±4.3% of mRNA reads (C is 57% lower than T; Welch Two Sample t-test, $p=8.3e^{-05}$), the first demonstration of altered RNA expression in human tissue linked to *SPINK1* p.Asn34Ser.[8]

Among heterozygous *PRSS1* p.Asn246= (rs6667 TC) individuals (n=12), the low-risk *PRSS1*_rs6667_T allele represented 47.5%±4.0% of mRNA reads and the high-risk allele *PRSS1*_rs6667_C represented 52.5%±4.0% of mRNA reads (C is 5% higher than T; Wilcoxon signed ranked test, p=0.021). High variability in expression suggests that independent variants also affect *PRSS1* expression. Thus, low expression of the *SPINK1*_rs17107315_C (p.Ser34) explains increased RAP/CP risk[8], but the minimal increase in *PRSS1* expression from *PRSS1*_rs6667_C [risk] cannot explain the higher risk of the *PRSS1-PRSS2* risk haplotype.

The relative expression of *PRSS1* and *SPINK1* among different pancreatic cell subtypes was evaluated using scRNA-Seq. A heatmap identified 10 major cell types using 10 cell marker genes to classify each cell type (Figure S1). A large fraction of cells were partially undifferentiated acinar-type cells[9] with some genes from other cell types expressed at low levels (Figure S1, column 2 [undifferentiated cells]; and column 4 [acinar cells]). Figure 1A is a UMAP plot of all cell types. Expression of *PRSS1* and *SPINK*1 in cell types is shown by feature plots (Figure 1B-C). The ratio of *PRSS1/SPINK1* ranged from 5.92 to 7.38 with an average of 6.47, which is slightly lower than the *PRSS1/SPINK1* ratio of 18.5 ratio from GTEx. (https://gtexportal.org/home/eqtls/tissue?tissueName=Pancreas accessed 8/18/21). The slightly higher *SPINK1* levels in our samples may be from underlying inflammation.[10]

The ratio of *PRSS1/SPINK* was high in subsets of undifferentiated acinar-type cells (circled in Figure 1A, expanded in 1D into 9 subgroups [0 to 8]). Violin plot showed the *PRSS1* and *SPINK1* expression level in subclusters (Figure 1E). The *PRSS1*/*SPINK1* expression ratio was highest in sub-clusters 5 and 6 (Figure 1F). KEGG pathway enrichment in sub-cluster 6 indicated that cells with high *PRSS1/SPINK1* ratios also had marked inflammation and immune gene expression responses, consistent with unregulated trypsin activity and

cell injury versus acinar cells (Supplemental Table S1). This indicates that cells with low *SPINK1* relative to *PRSS1* expression are undergoing intracellular damage, likely related to uninhibited trypsin activity.[7]

Thus, SPINK1 protects the pancreas by inhibiting active PRSS1 (trypsin). Low *SPINK1* expression is a risk for cell injury and RAP/CP. The *PRSS1-PRSS2* risk haplotype has little effect on *PRSS1* expression or interaction with *SPINK1.* The *PRSS1-PRSS2* risk haplotype is strongly associated with altered expression of *TRBV29–1* and other *TRB* transcripts. This data suggests that the strong risk of the *PRSS1-PRSS2* haplotype for RAP/CP may be through variant *TRB* repertoires that alter immune phenotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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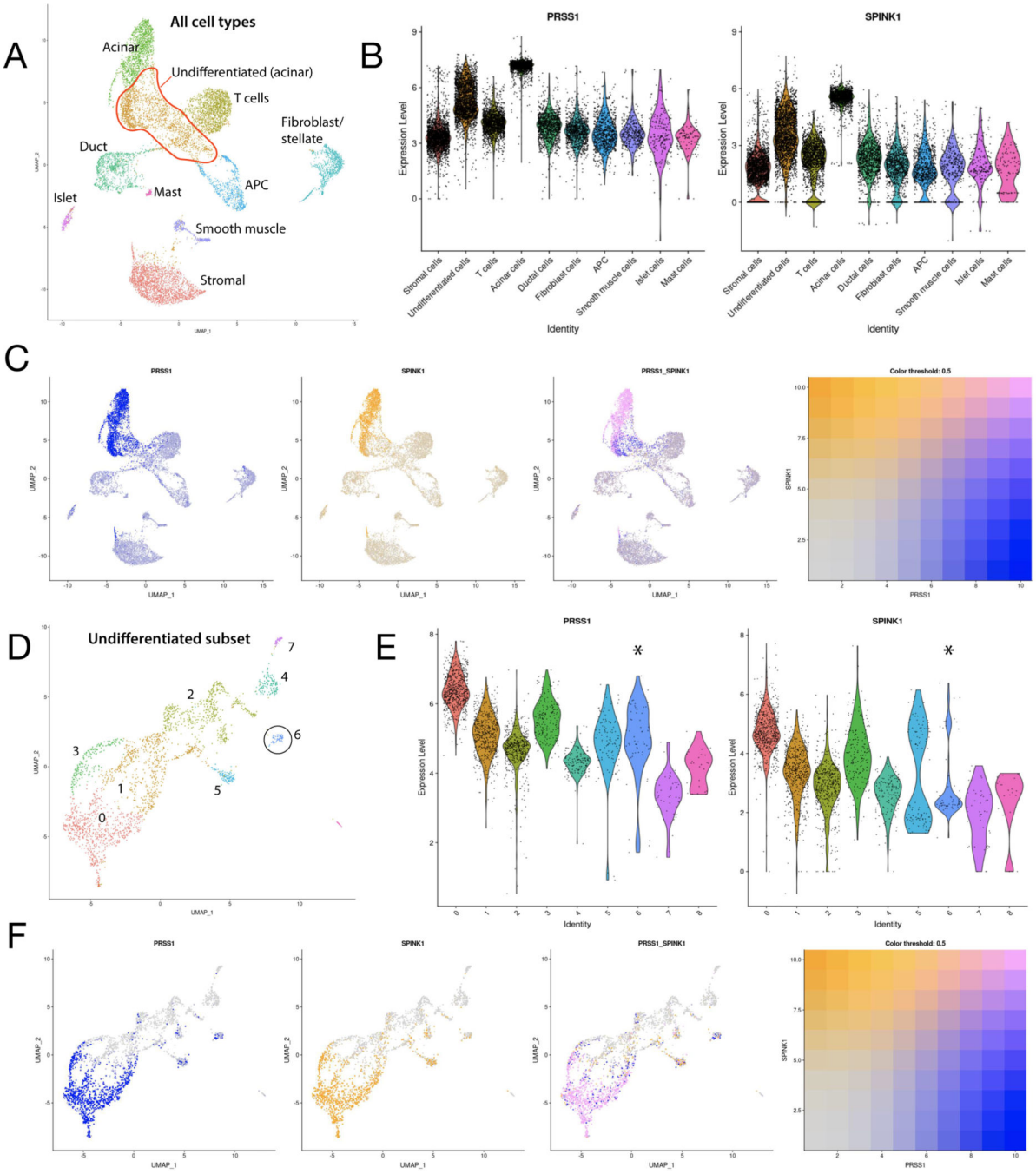

Figure 1.

PRSS1 and *SPINK1* expression profile. (A) UMAP of all pancreatic cell type clusters. (B) Violin plot of *PRSS1* and *SPINK1* in all pancreatic clusters. (C) Co-expression of *PRSS1* and *SPINK1* in all cell-clusters. (blue: *PRSS1* expression, orange: *SPINK1* expression, pink: co-expression) (D) UMAP of undifferentiated cells sub-cluster from D. Subclusters are numbered by cell amounts. Cluster 6 transcriptome analysis is in supplemental Table S1. (E) Violin plot of *PRSS1* and *SPINK1* in 9 clusters in undifferentiated cells (0–8).

*denotes cluster 6 *PRSS1* vs *SPINK1* expression. (F) Co-expression of *PRSS1* and *SPINK1* in undifferentiated cells from panel D.