SUPPLEMENTARY APPENDIX

Canakinumab Reverses Overexpression of Inflammatory Response Genes in Tumor Necrosis Factor Receptor–Associated Periodic Syndrome

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METHODS

Identification of Differentially Expressed Genes

To identify differentially expressed genes, samples were included in a linear regression model using the LIMMA package in R, with visit number and array batch as factors. Pairwise comparisons between different visits were performed to determine the differentially expressed genes using subject as the random effect. TRAPS patients at baseline were also contrasted with healthy volunteers. Differential expression was defined using a maximum Benjamini-Hochberg corrected P-value cutoff of 0.05 and a minimum 2-fold change in either direction. Because neutrophil counts were previously observed to decline during canakinumab treatment, the potential interaction between changes in neutrophil counts and changes in gene expression changes were evaluated. An analysis of variance (ANOVA) was first used to determine changes in neutrophil counts with canakinumab treatment across the visits at baseline, Day 15, and Day 113, and then Pearson's correlation was used to determine whether differentially expressed genes correlated with the relative neutrophil count.

Supplementary Table 1. *TNFRSF1A* Gene Mutations in the Study Cohort

***TNFRSF1A Gene Mutation*, n (%)**	**N=20**
T50M	4 (20)
C55Y	2 (10)
C33Y	2 (10)
C72N	2 (10)
C29Y	1 (5)
C52Y	1 (5)
D42del	1 (5)*
C30R	1 (5)
T371	1 (5)
F60L	1 (5)
Y38S	1 (5)
C43G	1 (5)
C72Y	1 (5)
C81Y	1 (5)

***Patient excluded from the gene expression analysis due to quality control.**

Supplementary Table 2. TRAPS Disease Signature Genes

Untreated TRAPS patients at baseline were compared to healthy volunteers to identify differentially expressed genes. Genes with an absolute fold change >2 and an adjusted p-value <0.05 were used to define a TRAPS disease gene signature. Genes with gene ontology annotation are listed.

Gene Symbol	Gene Name	Relative Change
AFF1	af4/fmr2 family member 1	Up
AGL	glycogen debranching enzyme	Down
AKR1C3	aldo-keto reductase family 1 member c3	Down
ATP9A	phospholipid-transporting atpase iia-related	Up
BPI	bactericidal permeability-increasing protein	Up
C5orf28	transmembrane protein c5orf28	Down
CD160	cd160 antigen	Down
CD69	early activation antigen cd69	Down
CLU	clusterin	Up
COMMD8	comm domain-containing protein 8	Down
COX6C	cytochrome c oxidase subunit 6c	Down
COX7B	cytochrome c oxidase subunit 7b, mitochondrial	Down
COX7C	cytochrome c oxidase subunit 7c, mitochondrial	Down
CPEB4	cytoplasmic polyadenylation element-binding protein 4	Up
CYP1B1	cytochrome p450 1b1	Up

EIF1AX	eukaryotic translation initiation factor 1a, x-chromosomal	Down
F5	coagulation factor v	Up
FCER1A	high affinity immunoglobulin epsilon receptor subunit alpha	Down
FFAR2	free fatty acid receptor 2	Up
FOLR3	folate receptor gamma	Up
FPR2	n-formyl peptide receptor 2	Up
GLMN	glomulin	Down
HIP1	huntingtin-interacting protein 1	Up
HNRNPL	heterogeneous nuclear ribonucleoprotein l	Up
HOPX	homeodomain-only protein	Down
ITGB3BP	centromere protein r	Down
KDM5D	lysine-specific demethylase 5d	Up
KLRB1	killer cell lectin-like receptor subfamily b member 1	Down
KLRF1	killer cell lectin-like receptor subfamily f member 1	Down
LILRA5	leukocyte immunoglobulin-like receptor subfamily a member 5	Up
LSM3	u6 snrna-associated sm-like protein lsm3	Down
MAPK14	mitogen-activated protein kinase 14	Up
MMP9	matrix metalloproteinase-9	Up
MRPL1	39s ribosomal protein l1, mitochondrial	Down
MRPL3	39s ribosomal protein l3, mitochondrial	Down
MYL9	myosin regulatory light polypeptide 9	Up
NDUFA5	nadh dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5	Down

NELL2	protein kinase c-binding protein nell2	Down
NOC3L	nucleolar complex protein 3 homolog	Down
NOP58	nucleolar protein 58	Down
OSTF1	osteoclast-stimulating factor 1	Up
PFDN5	prefoldin subunit 5	Down
PLIN3	perilipin-3	Up
PLSCR1	phospholipid scramblase 1	Up
PMS1	pms1 protein homolog 1	Down
PXK	px domain-containing protein kinase-like protein	Up
ROPN1L	ropporin-1-like protein	Up
RPS24	40s ribosomal protein s24	Down
RPS4Y1	40s ribosomal protein s4, y isoform 1	Up
RSL24D1	ribosome biogenesis protein rlp24-related	Down
RWDD1	rwd domain-containing protein 1	Down
RWDD3	rwd domain-containing protein 3	Down
SH3YL1	sh3 domain-containing ysc84-like protein 1	Down
SIPA1L2	signal-induced proliferation-associated 1-like protein 2	Up
SRPK1	srsf protein kinase 1	Up
TC2N	tandem c2 domains nuclear protein	Down
TLR5	Toll-like receptor 5	Up
TMCO3	transmembrane and coiled-coil domain-containing protein 3	Up
TMEM176A	transmembrane protein 176a	Up

TOMM7	mitochondrial import receptor subunit tom7 homolog	Down
TREML1	trem-like transcript 1 protein	Up
TRMT13	trna:m(4)x modification enzyme trm13 homolog	Down
USF1	upstream stimulatory factor 1	Up
ZMAT1	zinc finger matrin-type protein 1	Down
ZNF14	zinc finger protein 14	Down
ZNF302	zinc finger protein 135-related	Down
ZNF600	zinc finger protein 600	Down
ZNF675	zinc finger protein 675	Down
ZNF816	zinc finger protein 816	Down
ZNF83	zinc finger protein 83-related	Down

Supplementary Table 3. Canakinumab Treatment Signature Genes.

Canakinumab-treated TRAPS patients at Day 15 were compared to untreated TRAPS patients at baseline to identify differentially expressed genes. Genes with an absolute fold change >2 and an adjusted p-value <0.05 were used to define a canakinumab disease gene signature. Genes with gene ontology annotation are listed.

Gene Symbol	Gene Name	Relative Change
ADM	adm	Down
ANXA3	annexin a3	Down
ASGR2	asialoglycoprotein receptor 2	Down
CD69	early activation antigen cd69	Up
CLEC4D	c-type lectin domain family 4 member d	Down
CR1	complement component receptor 1-like protein-related	Down
CYP1B1	cytochrome p450 1b1	Down
CYSTM1	cysteine-rich and transmembrane domain-containing protein 1	Down
DGAT2	diacylglycerol o-acyltransferase 2	Down
DYSF	dysferlin	Down
F5	coagulation factor v	Down
FFAR2	free fatty acid receptor 2	Down
FOLR3	folate receptor gamma	Down
GOLGA8A	golgin subfamily a member 8a-related	Up

GPR97	g-protein coupled receptor 97-related	Down
HK3	hexokinase-3	Down
LILRA5	leukocyte immunoglobulin-like receptor subfamily a member 5	Down
LIMK2	lim domain kinase 2	Down
LRG1	leucine-rich alpha-2-glycoprotein	Down
MAPK14	mitogen-activated protein kinase 14	Down
MMP9	matrix metalloproteinase-9	Down
MRVI1	protein mrvi1	Down
ORM1	alpha-1-acid glycoprotein 1-related	Down
PGLYRP1	peptidoglycan recognition protein 1	Down
RFX2	dna-binding protein rfx2	Down
ROPN1L	ropporin-1-like protein	Down
S100A12	protein s100-a12	Down
SIPA1L2	signal-induced proliferation-associated 1-like protein 2	Down
SLC22A4	solute carrier family 22 member 4	Down
SLPI	antileukoproteinase	Down
SRPK1	srsf protein kinase 1	Down
SULT1B1	sulfotransferase family cytosolic 1b member 1	Down
TC2N	tandem c2 domains nuclear protein	Up
TECPR2	tectonin beta-propeller repeat-containing protein 2	Down
TLR5	toll-like receptor 5	Down
TRAT1	t-cell receptor-associated transmembrane adapter 1	Up

TRMT13	trna:m(4)x modification enzyme trm13 homolog	Up
VNN1	pantetheinase	Down
WDFY3	wd repeat and fyve domain-containing protein 3	Down
ZMAT1	zinc finger matrin-type protein 1	Up
ZNF302	zinc finger protein 135-related	Up
ZNF600	zinc finger protein 600	Up