Supplementary data

The circulating T helper subsets and regulatory T cells in patients with common variable immunodeficiency with no monogenic disease

Azizi et al.

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Table S1. Excluded 373 genes variants in whole exome sequencing of CVID patients.

Genes symbols							
ACP5	*CD27*	*DEPTOR*	*IGHA2*	*LIG4*	*NOLA2*	*ROBLD3*	*TLR3*
ACT1	*CD28*	*DGCR*	*IGHE*	*LPIN2*	*NOLA3*	*RPSA*	*TMC6*
ACTB	*CD3D*	*DKC1*	*IGHG1*	*LRBA*	*NOP10*	*RORC*	*TMC8*
ADA	*CD3E*	*DNMT3B*	*IGHG2*	*LRRC8A*	*NOTCH1*	*RSPA*	*TMEM173*
ADAR1	*CD3G*	*DOCK2*	*IGHG3*	*LYST*	*PNP*	*RTEL1*	*TNFAIP3*
ADAM17	*CD40*	*DONSON*	*IGHG4*	*MAP3K14*	*NRAS*	*RLTPR*	*TNFRSF11A*
AICDA	*CD40LG*	*DOCK8*	*IGHM*	*MAGT1*	*NSMCE3*	*RTP4*	*TNFRSF13B*
AIRE	*CD46*	*DRB1*	*IGKC*	*MALT1*	*ORAI1*	*SAMHD1*	*TNFRSF13C*
AK2	*CD55*	*E47*	*IGLL1*	*MASP1*	*OX40*	*SBDS*	*TNFRSF1A*
AP3B1	*CD59*	*ELANE*	*IKAROS*	*MASP2*	*PARN*	*SEC16A*	*TNFRSF6*
AP3D	*CD70*	*ELF4*	*IKBA*	*MBL2*	*PAX5*	*SERPING1*	*TNFSF6*
APOL1	*CD79A*	*EPG5*	*IKBKB*	*MCM10*	*PGM3*	*SH2D1A*	*TRAC*
ARPC1B	*CD79B*	*EVER1*	*IKBKG*	*MCM4*	*PIGA*	*SH3BP2*	*TRAF3*
ATM	*CD81*	*EVER2*	*IKZF1*	*MDA5*	*PIK3CD*	*SLC11A1*	*TREX1*
BCL10	*CD8A*	*EVER3*	*IL10*	*MEFV*	*PIK3R1*	*SLC29A3*	*TRNT1*
BLM	*CDCA7*	*F12*	*IL10RA*	*mirc1*	*PLCG2*	*SLC35C1*	*TTC37*
BLNK	*CEBPE*	*FADD*	*IL10RB*	*MKL1*	*PLDN*	*SLC37A4*	*TTC7A*
BTK	*CECR1/ADA2*	*FANCA*	*IL12B*	*MLPH*	*PMM2*	*SLC46A1*	*TWEAK*
B2M	*CFB*	*FANCE*	*IL12RB1*	*MOGS*	*PMS2*	*SMC5/6*	*TYK2*
C16orf57	*CFD*	*FAS*	*IL17F*	*MPO*	*POLE1*	*SMARCAL1*	*TPP1*
C1QA	*CFH*	*FASLG*	*IL17RA*	*MRE11A*	*PRF1*	*SP110*	*TPP2*
C1QB	*CFHR1*	*FCGR1A*	*IL1RN*	*MSH6*	*PRKCD*	*SPINK5*	*UNC119*
C1QC	*CFHR3*	*FCGR3A*	*IL21*	*MST1*	*PRKDC*	*STAT1*	*UNC13D*
C1R	*CFHR5*	*FCN3*	*IL21R*	*MSN*	*PSMB8*	*STAT2*	*UNC93B1*
C1S	*CFI*	*FERMT3*	*IL23*	*MTHFD1*	*PTEN*	*STAT3*	*UNG*
C2	*CFP*	*FH*	*IL2RA*	*MUC2*	*PSTPIP1*	*STAT5B*	*VAV1*
C3	*CHD7*	*FH3*	*IL2RG*	*CD20*	*PTPRC*	*STIM1*	*VPREB1*
C4A	*CIAS1*	*FOXN1*	*IL36RN*	*MVK*	*RAB27A*	*STK4*	*VPS13B*
C4B	*CIITA*	*FOXP3*	*IL7R*	*MYD88*	*RAC2*	*STN1*	*VPS45*
C4BPA	*CLEC16A*	*FPR1*	*INO80*	*MYO5A*	*RAG1*	*STX11*	*WAS*
C4BPB	*CLEC7A*	*FUCT1*	*IRAK4*	*NBN*	*RAG2*	*STXBP2*	*WASF2*
C5	*CLPB*	*G6PC*	*IRF8*	*NBS1*	*RASGRP1*	*SEC61A1*	*WIPF1*
C6	*CMCD7*	*G6PC3*	*IRF7*	*NCF1*	*RASGRP2*	*SPPL2A*	*WRAP53*
C7	*CMCD8*	*G6PD*	*IRFBP2*	*NCF2*	*RBCK1*	*TAP1*	*XIAP*
C8A	*COH1*	*G6PT1*	*ISG15*	*NCF4*	*RC3H1*	*TAP2*	*ZAP70*
C8B	*COLEC11*	*GATA2*	*ITCH*	*NEIL1*	*RECQL4*	*TAPBP*	*ZBTB24*
C8G	*CORO1A*	*GFI1*	*ITGA3*	*NEIL3*	*RFX5*	*TAZ*	*ZNF341*
C9	*COPA*	*GIAMP5*	*ITGB2*	*NFAT5*	*RFXANK*	*TBK1*	
CARD11	*CSF2RA*	*HAX1*	*ITK*	*NFKB1*	*RFXAP*	*TBX1*	
CARD14	*CSF3R*	*HELLS*	*JAGN1*	*NFKB2*	*RHOH*	*TCF3*	
CARD9	*CTLA4*	*HOIL1*	*JAK2*	*NFKBIA*	*RIPK1*	*TCIRG1*	
CASP10	*CTSC*	*HOIP*	*JAK3*	*NFKBID*	*RLTPR*	*TCN2*	
CASP8	*CTPS1*	*ICOS*	*KMT2A*	*NHEJ1*	*RMRP*	*TERC*	
CCBE1	*CXCR4*	*IFNGR1*	*KINDLIN3*	*NHP2*	*RNASEH2A*	*TERT*	
CD16	*CYBA*	*IFNGR2*	*KRAS*	*NLRP12*	*RNASEH2B*	*TFRC*	
CD19	*CYBB*	*IFIH1*	*LAT*	*NLRC4*	*RNASEH2C*	*THBD*	
CD21	*DCLRE1B*	*IGAD1*	*LCK*	*NLRP3*	*RNU4ATAC*	*TICAM1*	
CD247	*DCLRE1C*	*IGHA1*	*LIG1*	*NOD2*	*RNF168*	*TINF2*	

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Table S2. Oligonucleotide sequences of primers used in this study for quantitative real time PCR.

Genes	Forward	Reverse
IFNG	F; 5'-GAGTGTGGAGACCATCAAGGAA-3'	R; 3'-GGCGACAGTTCAGCCATCA-5'
IL17	F; 5'-CTACAACCGATCCACCTCACC-3'	R; 3'-CCCACGGACACCAGTATCTTC-5'
IL22	F; 5'-CCTTGAAGAAGTGCTGTTCCCT-3'	R; 3'-CCTTCAGCTTTTGCACATTCCT-5'
IL10	F; 5'-GACTTTAAGGGTTACCTGGGTTG-3'	R; 3'-TCTTGGTTCTCAGCTTGGGG-5'
TBET	F; 5'-GTCGCGCTCAACAACCAC-3'	R; 3'-GGAACATCCGCCGTCCCT-5'
RORC	F; 5'-TTCCGAGGATGAGATTGCCC-3'	R; 3'-CAGCTTTGCCAGGATGCTTT-5'
AHR	F; 5'-GTCGTCTAAGGTGTCTGCTGG-3'	R; 3'-TATGGATGGTGGCTGAAGTGG-5'
FOXP3	F; 5'-ACAACATGCGACCCCCTTT-3'	R; 3'-TGGCGGATGGCGTTCTTC-5'
RUNX1	F; 5'-AATGCTACCGCAGCCATGAA-3'	R; 3'-CTTGCGGTGGGTTTGTGAAG-5'
GAPDH	F; 5'-GAGAAGGCTGGGGCTCATTT-3'	R; 3'-TAAGCAGTTGGTGGTGCAGG-5'

Table S3. The absolute counts of the investigated lymphocyte subsets in CVID patients and Healthy controls.

Subset; cell/µL	CVID patients (n=13)	Healthy controls (n=13)	*P* value
Lymphocyte($\times 10^3$)	2.00 (1.30-2.70)	2.20 (180-2.40)	0.459
CD4+ T cells	567 (359-750)	936 (842-1027)	0.003*
$CD4^+$ IFN-γ^+ IL-17^- (Th1)	72 (29-176)	112 (101-143)	0.174
$CD4^+$ IFN-γ^+ IL-17^+ (Th1-like Th17)	4.66 (1.21-6.29)	4.62 (3.62-5.92)	0.596
$CD4^+$ IFN-γ^- IL-17^+ (Th17)	1.50 (0.46-4.10)	5.92 (4.89-7.67)	0.001*
$CD4^+$ IFN-γ^-IL-17^- IL-22^+ (Th22)	4.68 (0.99-13.48)	4.88 (3.69-5.92)	0.800
$CD4^+$ $CD25^+$ $FoxP3^+$ (Treg)	3.47 (1.76-10.11)	19.40 (14.62-22.90)	<0.001*
$CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{low/-}$ ($CD127^{low-}$Treg)	2.40 (1.05-12.19)	17.69 (12.55-22.48)	<0.001*

Data are presented as median (75^{th}-25^{th} interquartile range).
**P values <0.05 were regarded significant.*

Figure S1.

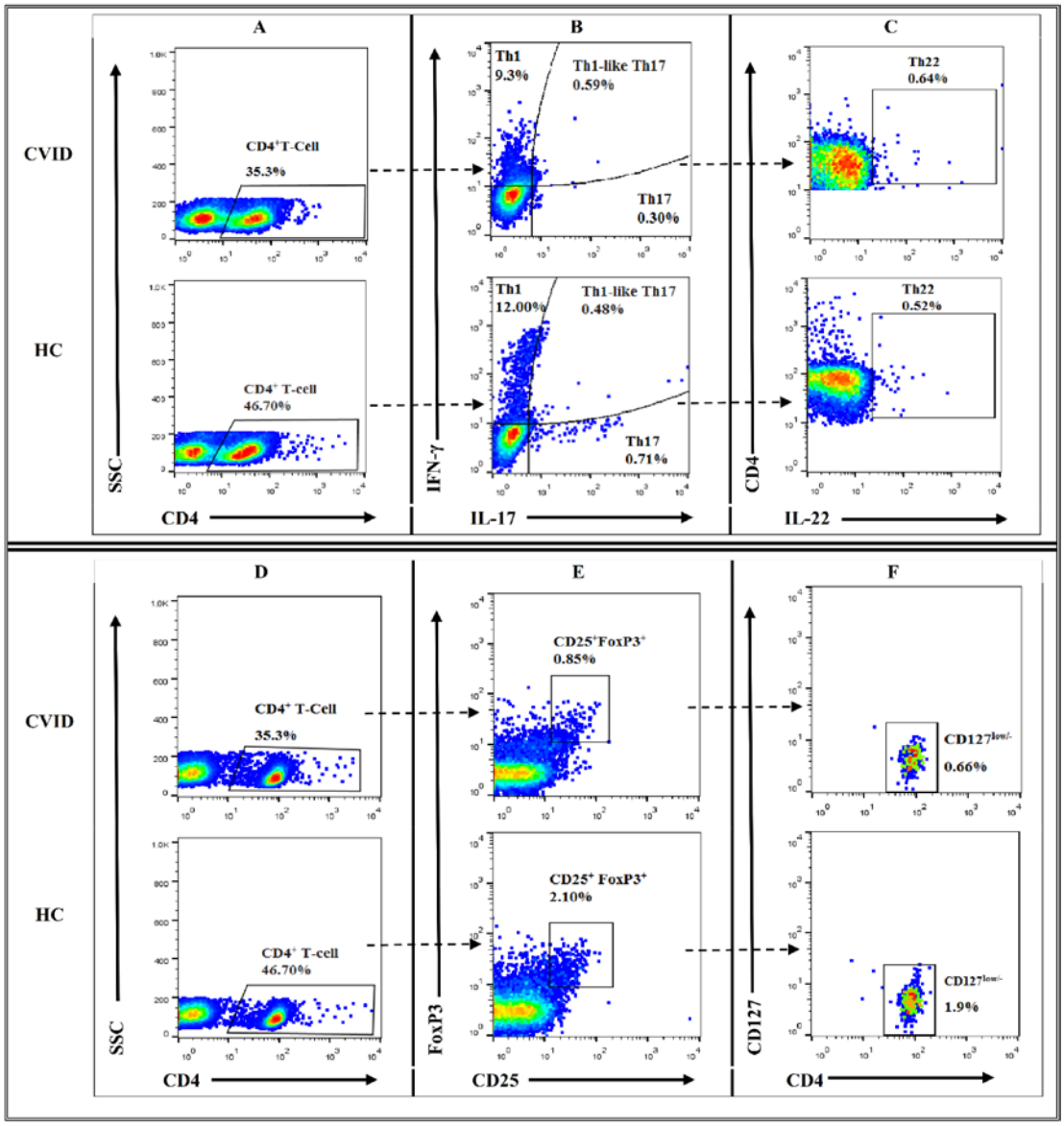

Figure S1. Flow cytometry analysis of different subsets of $CD4^+$ T cells. Two examples demonstrate the flow cytometric analysis of peripheral blood samples (PBMCs) from one CVID patient and a healthy control (HC). (**A-C**) Th subsets: PBMCs from subjects were prepared and cultured with PMA and ionomycin for 5 hours, and then cells were harvested. The cells were stained with anti-CD4 PerCP-cy5.5, fixed, and permeabilized, followed by intracellular staining with anti-IFN-γ FITC, anti-IL-17 PE, and anti-IL-22 APC. $CD4^+$ T-cells were gated within the lymphocyte scatter region (**A**) for analysis of the $CD4^+$ $IFN\gamma^+$ $IL\text{-}17^-$, $CD4^+$ $IFN\gamma^+$ $IL\text{-}17^+$, $CD4^+$ $IFN\gamma^-$ $IL\text{-}17^+$ cells (**B**). The $CD4^+$ $IFN\gamma^-$ $IL\text{-}17^-$ cells were further analyzed for $CD4^+$ $IFN\gamma^-$ $IL\text{-}17^-$ $IL\text{-}22^+$ cells (**C**). (**D-F**) Treg analysis: PBMCs were isolated and stained with anti-CD4 PerCP-cy5.5, anti-CD25 APC, anti-CD127 FITC, fixed, and permeabilized, followed by intracellular staining with anti-Foxp3 PE. Then, the cells were gated first on $CD4^+$ cells for analysis of the $CD4^+$ $CD25^+$ $FoxP3^+$. $CD4^+$ $CD25^+$ $FoxP3^+$ cells were further analyzed for $CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{low/-}$ cells. Data are expressed as the median values of individual participants. Arrows indicate the gated population subsequently analyzed.