

Supplementary data

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

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	LRRRC8A	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	mircl	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	FOXP1	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	

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

Genes	Forward	Reverse
<i>IFNG</i>	F; 5'-GAGTGTGGAGACCATCAAGGAA-3'	R; 3'-GGCGACAGTTCAGCCATCA-5'
<i>IL17</i>	F; 5'-CTACAACCGATCCACCTCACC-3'	R; 3'-CCCACGGACACCAGTATCTTC-5'
<i>IL22</i>	F; 5'-CCTTGAAGAAGTGCTGTTCCCT-3'	R; 3'-CCTTCAGCTTTTGCACATTCCT-5'
<i>IL10</i>	F; 5'-GACTTTAAGGGTTACCTGGGTTG-3'	R; 3'-TCTTGGTTCTCAGCTTGGGG-5'
<i>TBET</i>	F; 5'-GTCGCGCTCAACAACCAC-3'	R; 3'-GGAACATCCGCCGTCCCT-5'
<i>RORC</i>	F; 5'-TTCCGAGGATGAGATTGCCC-3'	R; 3'-CAGCTTTGCCAGGATGCTTT-5'
<i>AHR</i>	F; 5'-GTCGTCTAAGGTGTCTGCTGG-3'	R; 3'-TATGGATGGTGGCTGAAGTGG-5'
<i>FOXP3</i>	F; 5'-ACAACATGCGACCCCTTT-3'	R; 3'-TGGCGGATGGCGTTCTTC-5'
<i>RUNX1</i>	F; 5'-AATGCTACCGCAGCCATGAA-3'	R; 3'-CTTGCGGTGGGTTTGTGAAG-5'
<i>GAPDH</i>	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/ μ L	CVID patients (n=13)	Healthy controls (n=13)	P value
Lymphocyte($\times 10^3$)	2.00 (1.30-2.70)	2.20 (1.80-2.40)	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 (75th-25th interquartile range).

*P values <0.05 were regarded significant.

Figure S1.

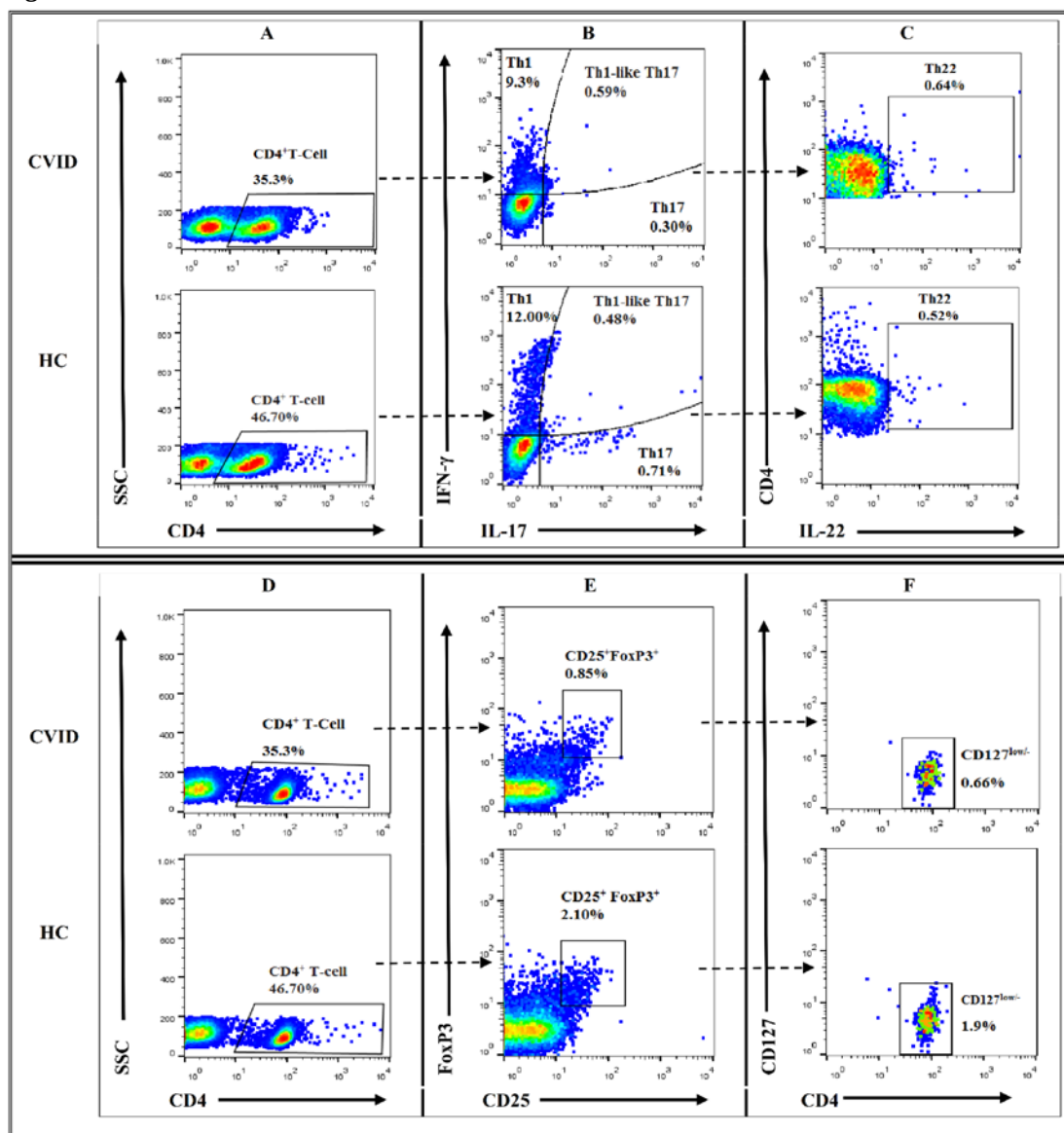


Figure S1. Flow cytometry analysis of different subsets of CD4⁺ T cells. Two examples demonstrate the flow cytometric analysis of peripheral blood samples (PBMCs) from one CVID patient and a healthy control (HC). (A-C) Th subsets: PBMCs from subjects were prepared and cultured with PMA and ionomycin 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- γ ⁺ IL-17⁻, CD4⁺ IFN- γ ⁺ IL-17⁺, CD4⁺ IFN- γ ⁻ IL-17⁺ cells (B). The CD4⁺ IFN- γ ⁻ IL-17⁺ cells were further analyzed for CD4⁺ IFN- γ ⁻ IL-17⁺ IL-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.