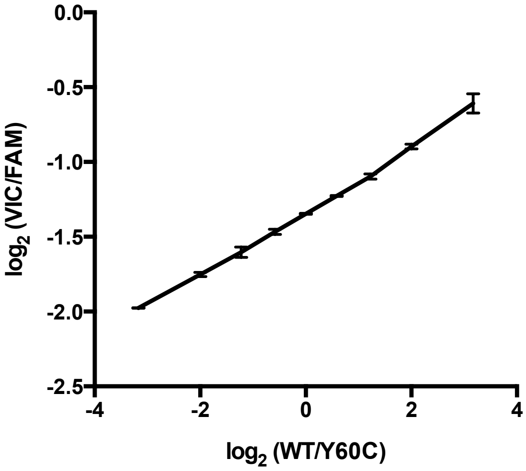
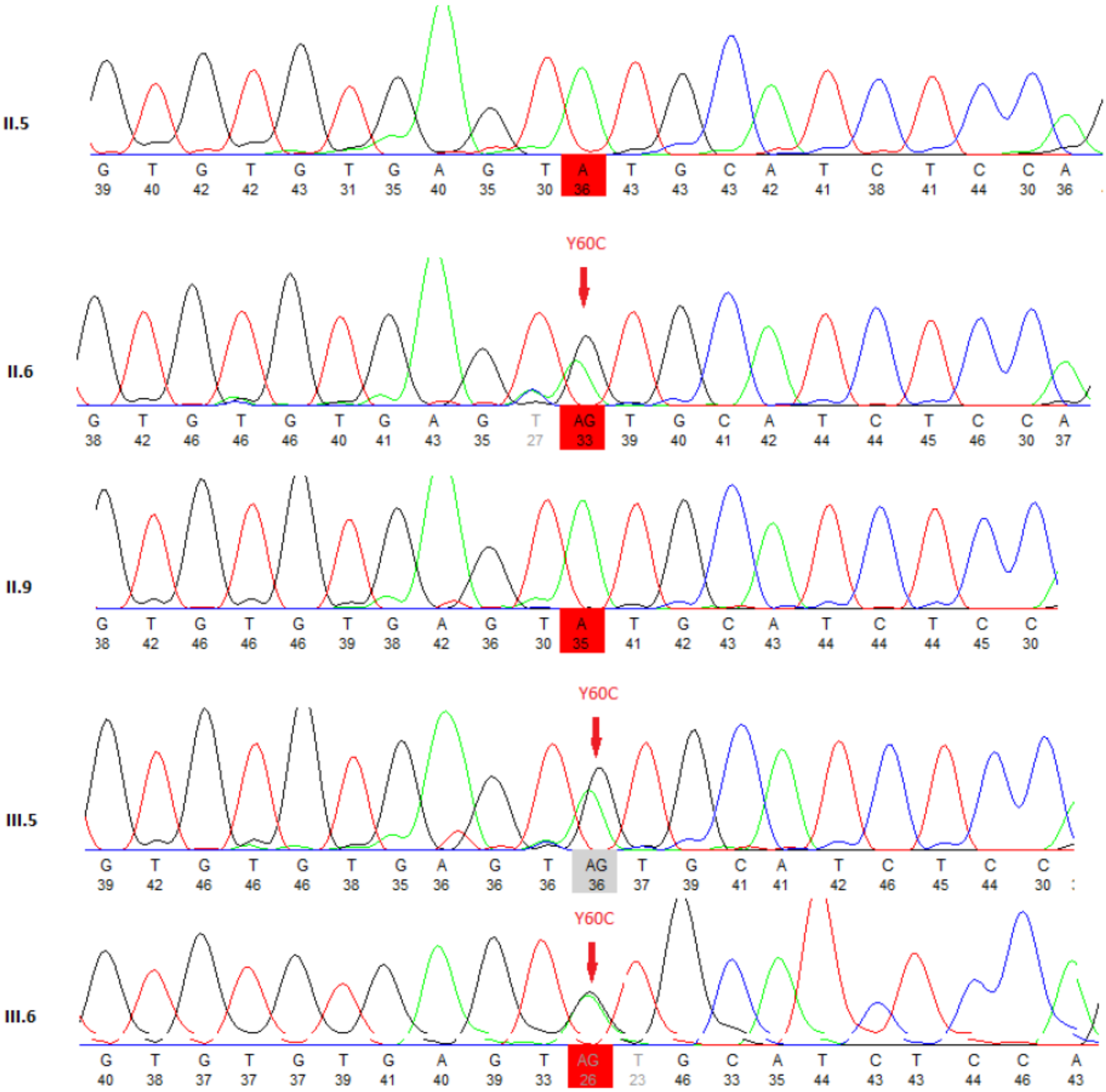
Suppl. Fig. 1



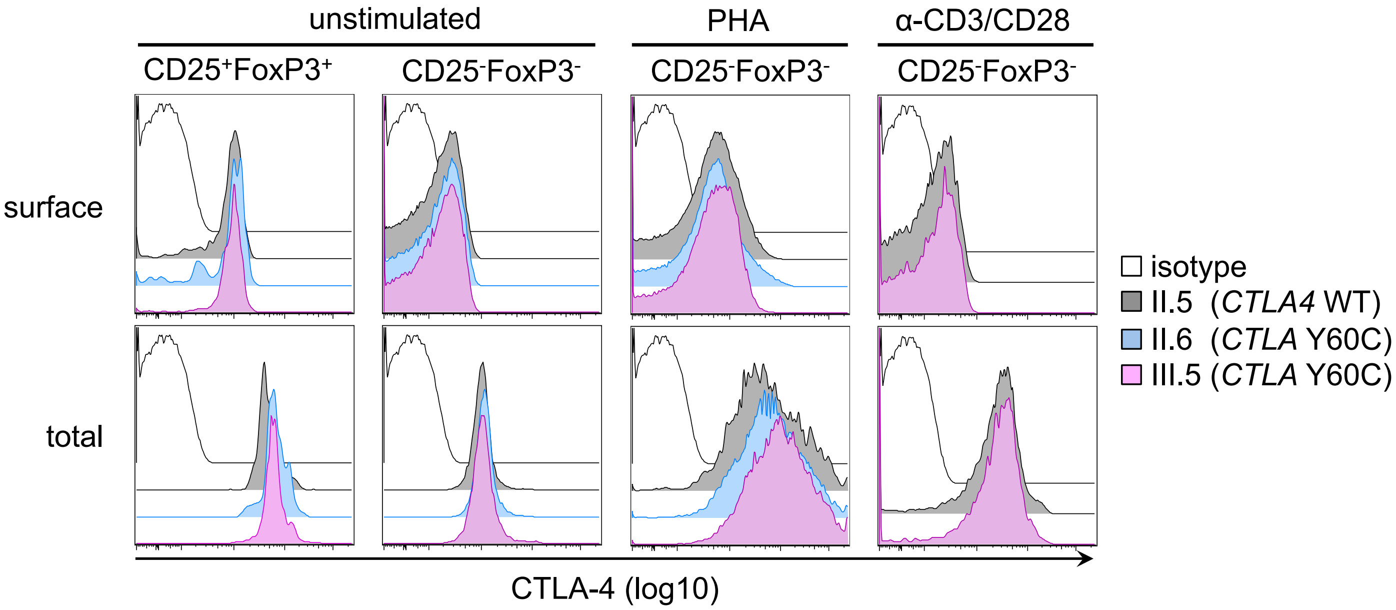
Suppl. Fig. 1. DNA from WT and Y60C CTLA-4 constructs (see Methods) were mixed so that the mix contained between 10 and 90% of WT and Y60C DNA. The figure demonstrates that the log2 value of this ratio has a linear relationship with the ratio of the log2 of the ratio of the VIC to FAM signal observed with a Taqman genotyping assay. The ratio between the VIC and FAM signal was then used to determine the allele specific expression in samples from the family.

Suppl. Fig. 2



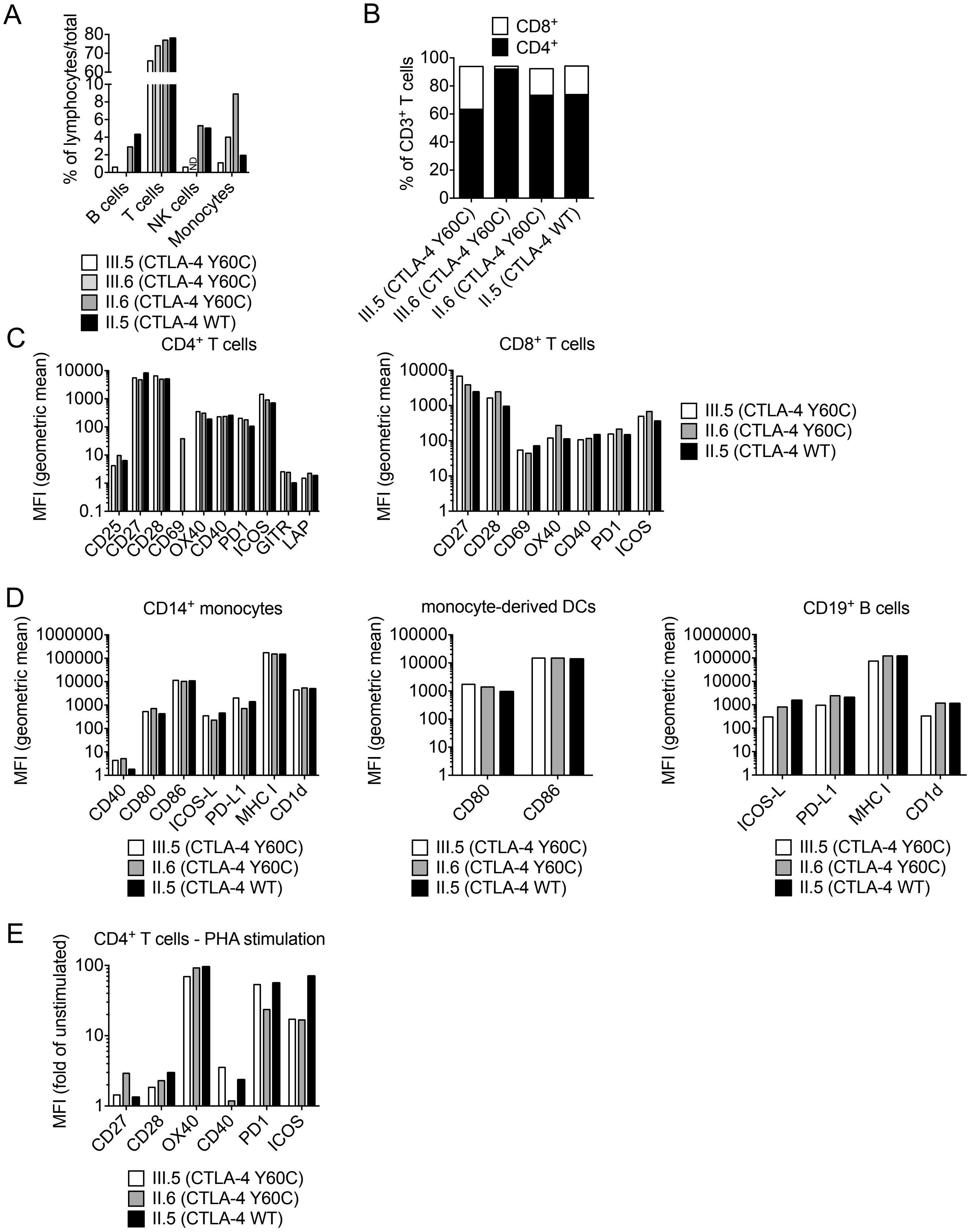
Suppl. Fig. 2. Sanger sequencing traces of the affected family showing the presence or absence of *CTLA4* c.179A>G.

Suppl. Fig. 3



Suppl. Fig. 3. Unaltered expression of CTLA-4 Y60C by primary T cells. Cell surface and total (surface and intracellular) expression of CTLA-4 by CD3$^+$ CD4$^+$ CD25$^+$ FoxP3$^+$ Tregs and CD3$^+$ CD4$^+$ CD25$^-$ FoxP3$^-$ T cells as determined by flow cytometry either directly *ex vivo* (unstimulated) or after activation by anti-CD3/CD28 or PHA.

Suppl. Fig. 4



Suppl. Fig. 4. Phenotype and function of immune cells in carriers of WT and Y60C CTLA-4. A. Percentage of the indicated cell populations among lymphocytes (T, B, NK cells) or total cells (monocytes) as determined by flow cytometry. B. Percentage of $CD4^+$ and $CD8^+$ cells among $CD3^+$ T cells as determined by flow cytometry. C. Expression of selected cell surface markers by $CD4^+$ T cells (left) and $CD8^+$ T cells (right) as determined by flow cytometry. D. Expression of selected cell surface markers as determined by flow cytometry on monocytes (left), monocyte-derived DCs (middle) and B cells (right). E. Fold induction of selected cell surface markers on $CD4^+$ T cell upon stimulation by PHA as determined by flow cytometry.

Suppl. Table. 1

Suppl. Table 1. Primer sequences and conditions for Sanger sequencing of *CTLA4*

primer name	primer sequence	T_M
CTLA-4_Exon1_F	CACGGCTTCCTTTCTCGTAA	60
CTLA-4_Exon1_R	AGGGTCTGAAATTGCCTGTG	60
CTLA-4_Exon2_F	GAGAGGGGAAGGGGTAAGTG	60
CTLA-4_Exon2_R	AGACTGCAATGCAACAGGTG	60
CTLA-4_Exon3_F	CCAAACCTATTGGTGGGCTA	60
CTLA-4_Exon3_R	GAAACCCTGCTCAGAAGCAC	60
CTLA-4_Exon4_F	ATAGGCTTTCTGGGTGTTCTTTC	60
CTLA-4_Exon4_R	GTGCCATTCCCTAACTTAATTCC	60
CTLA-4_pro1_F	CTGTTTGCATGTCAGCCTTC	60
CTLA-4_pro1_R	CTTCTGGGCATCCTTAACCA	60
CTLA-4_pro2_F	TTTCTCTTAACCAAATGCTAAATGG	60
CTLA-4_pro2_R	GTCCTGGTAGCCAGGTTCAG	60

Suppl. Table. 2

Suppl. Table 2. Exome Sequencing coverage

Sample	Coverage of exome target				
	% covered ≥1x	% covered ≥8x	% covered ≥20x	mean coverage	Gb of coverage
III.5	96.65	89.45	78.6	52.44	3.25
III.6	95.96	87.38	73.49	39.18	2.43
II.5	97.29	91.42	80.90	48.87	3.03
II.6	97.07	91.02	79.81	46.92	2.91
II.9	97.04	91	80.33	47.7	2.96

Suppl. Table. 3

Suppl. Table 3. Expression pattern of WT and Y60C CTLA-4 alleles.

	$\log_2$ (VIC/FAM)	Ratio WT/Y60C
Healthy control 1	0.22	150.4
Healthy control 2	0.23	157.3
II.6	-1.38	0.85
II.5	0.19	135.8
III.5	-1.28	1.19

Suppl. Table. 4

Suppl. Table 4. Results of *CTLA4* sequencing.

rsID	Genomic position (Build 36)	Variant	Function	II.9	II.5	II.6	III.6	III.5
rs231775	204,440,959	A>G	T17A	AG	AG	AG	AG	AG
n/a	204,443,623	A>G	Y60C	AA	AA	AG	AG	AG
rs59665421	204,446,337	T>C	3' UTR	TC	TT	TT	TT	TT

Suppl. Table. 5

Suppl. Table 5. Immunological characterization of patient III.6 under Ig supplementation.

Parameter	**Value**	**Reference**
IgG1 (mg/dl)	488	422-1,292
IgG2 (mg/dl)	307	117-747
IgG3 (mg/dl)	30	41-129
IgG4 (mg/dl)	<4	10-67
IgA (mg/dl)	8	70-312
IgM (mg/dl)	<4	40-240
White blood cell count (cells/µl)	3.43	5.82-9.32
Hemogloblin (g/dl)	11.6	10.9-13.4
Hematocrit (%)	36.6	31.7-39.1
Platelets (cells/µl)	266	174-333
Absolute neutrophil count (cells/µl)	2.5	3.32-6.3
Absolute lymphocyte count (cells/µl)	0.48	1.14-2.28
Absolute eosinophil count (cells/µl)	0	0.06-0.18
Neutrophils (%)	73	50-78
Lymphocytes (%)	14	10-38
Monocytes (%)	13	4-7
Eosinophils (%)	0	2-3
% CD3$^+$ (%)	85	56-84
CD3$^+$, absolute (cells/µl)	330	1,000-2,000
% CD3$^+$ CD4$^+$ (%)	56	28-52
CD3$^+$ CD4$^+$, absolute (cells/µl)	212	530-1,500
%CD3$^+$ CD8$^+$ (%)	26	18-35
CD3$^+$ CD8$^+$, absolute (cells/µl)	101	330-1,100
% CD3$^-$/CD16$^+$ or CD56$^+$ (%)	11	3-22
CD3$^-$/CD16$^+$ or CD56$^+$, absolute (cells/µl)	43	70-480
% CD19$^+$ (%)	1	6-23
CD19$^+$, absolute (cells/µl)	2	110-570
CD4$^+$/CD8$^+$ ratio	2.11	0.9-3.7

Suppl. Table. 6

Suppl. Table 6. RefSeq sequence identifiers for the sequences in Fig. 2A. Sequences are from human CTLA-4, orthologs from mammals, birds and reptiles, as well as three CD28 paralogs.

Species	**RefSeq identifier**
Homo sapiens	NP_005205
Oryctolagus cuniculus (European rabbit)	NP_001076154
Mus musculus (house mouse)	NP_033973
Sus scrofa (pig)	NP_999314
Felis catus (domestic cat)	NP_001009236
Bos taurus (cattle)	NP_776722
Loxodonta africana (African elephant)	XP_003406166
Monodelphis domestica (gray short-tailed opossum)	XP_001371314
Sarcophilus harrisii (Tasmanian devil)	XP_003766048
Gallus gallus (chicken)	NP_001035180
Taeniopygia guttata (zebra finch)	XP_002197489
Alligator mississippiensis (American alligator)	XP_006267305
Pelodiscus sinensis (Chinese softshell turtle)	XP_006114304
Homo sapiens (CD28)	NP_006130
Monodelphis domestica (CD28)	XP_007501953
Gallus gallus (CD28)	XP_990642

Suppl. Table. 7

Suppl. Table 7. Potential genetic modifiers. Exomes were filtered for SNVs in genes known to be associated with immunodeficiency, with a genotype shared between the affected children (III.5, III.6) but not with the unaffected mother (II.6), and excluding synonymous variants.

Chr	**Position**	**Gene symbol**	**Ref. Base**	**Var. Base**	**II.5**	**II.6**	**II.9**	**III.5**	**III.6**	**Mut. Type**	**Function**	**Effect**	**AA Subst**	**Phylo P44**	**Grant-ham**	**pph2 Prob.**	**SIFT score**	**Rs number**	**MAF G1000 CEU**
chr15	89,127,103	*BLM*	C	T	CT	CC	CT	CT	CT	het	Exon	mis	P868L	5.05	98	0.149	0.00	rs11852361	
chr15	89,155,525	*BLM*	G	A	AG	GG	AG	AG	AG	het	Exon	mis	V1321I	-0.39	29	0.003	0.61	rs7167216	
chr5	41,235,716	*C6*	G	T	GT	TT	GT	GT	GT	het	Exon	mis	A119E	1.38	107	0.001	1	rs1801033	0.675
chr2	86,922,293	*CD8B*	T	G	TT	GG	TT	GT	GT	het	Intron	-	-	-0.58	-		-	rs15800	
chr2	86,927,363	*CD8B*	C	T	CT	CC	CT	CT	CT	het	Exon	mis	V180I	0.36	29	0.155	1		
chr9	276,593	*DOCK8*	C	A	AA	CC	AC	AC	AC	het	Exon	mis	P29T	2.19	38	0.457	0.01	rs529208	0.416667
chr1	92,713,945	*GFI1*	A	G	AA	GG	AG	AG	AG	het	3UTR	-	-	-4.74	-		-	rs4970714	0.666667
chr1	205,008,152	*IL10*	T	C	CT	CC	TT	CT	CT	het	3UTR	-	-	-0.41	-		-	rs3024498	0.225
chr1	205,008,487	*IL10*	A	G	AG	GG	AG	AG	AG	het	3UTR	-	-	1.53	-		-	rs3024496	0.475
chr21	33,590,617	*IL10RB*	A	G	GG	AG	AG	GG	GG	hom	3UTR	-	-	-1.51	-		-	rs3171425	0.575
chr21	33,591,251	*IL10RB*	G	A	AA	AG	AG	AA	AA	hom	3UTR	-	-	-0.78	-		-	rs1058867	0.575
chr5	35,910,332	*IL7R*	C	T	CC	TT	CC	CT	CT	het	Exon	mis	T244I	-0.88	89	0.001	0.37	rs6897932	0.258333
chr11	57,138,565	*SERPING1*	G	A	GG	AA	GG	AG	AG	het	Exon	mis	V480M	-0.69	21	0.034	0.03	rs4926	0.216667
chr17	73,650,172	*TMC8*	A	G	GG	AA	GG	AG	AG	het	3UTR	-	-	0.34	-		-	rs4789015	0.516667
chr6	158,536,864	*GTF2H5*	A	C	AC	AA	AC	AC	AC	het	3UTR	-	-	-0.33	-		-	rs4354181	
chr6	158,539,936	*GTF2H5*	G	C	CG	GG	CG	CG	CG	het	3UTR	-	-	-0.48	-		-	rs71563797	0.158333
chr3	197,683,601	*RNF168*	G	T	GT	GT	GT	TT	TT	hom	Exon	mis	P401Q	1.68	76	0.216	0.01	rs3796129	0.591667
chr19	7,618,277	*STXBP2*	A	G	AA	GG	AG	AG	AG	het	Exon	mis	I526V	-0.48	29	0.000	0.58	rs6791	0.675