SUPPLEMENT

METHODS

*Sequencing***.** DNA was extracted from EDTA treated blood or from dried blood spots on filter cards (CentoCard®) using standard, spin column-based methods. Exome sequencing was performed as previously described (20). In short, the Nextera Rapid Capture Exome Kit (Illumina) or the SureSelect Human All Exon kit (Agilent) were used for enrichment, and a Nextseq500 or HiSeq4000 (Illumina) instrument was utilized for sequencing with the average coverage targeted to at least 100x or at least 98% of the target DNA covered 20x. Data analysis, including base calling, de-multiplexing, alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37), and variant calling, was performed using the HiSeq Analysis Software v2.0 pipelines (Illumina). Variants were annotated using SnpEff [21] and in-house bioinformatics tools [20].

Variants of suboptimal quality were confirmed via Sanger sequencing according to our established criteria (22). Forward and reverse primers were used for Sanger sequencing, on a 3730xl sequencer (Thermo Fisher Scientific). Variant nomenclature followed standard Human Genome Variation Society (HGVS) recommendations (23). Our data repository (CentoMD®) 17 contains ES/GS data from 60,234 individuals, of whom, 32,650 individuals have clinical descriptions that include at least one HPO term. After the initial identification of *CBLB* as a candidate gene, the data repository was queried for other rare variants in the gene and the clinical features and affection status of the individuals.

Cell preparation. Human PBMCs were isolated as previously described (24). EBV-transformed B cell lines from patients and controls were established as described (25).

Human T cell proliferation. $CD4^+CD25^-$ Teff cells were isolated from PBMCs using magnetic beads (Miltenyi). Teff cells were loaded with Cell Trace CFSE (Thermo Fisher) to monitor cell divisions and stimulated with anti-CD3 conjugated beads (Miltenyi) alone or with anti-CD28 (CD28.2, Biolegend). $CD4^+CD25^+$ Treg cells were similarly isolated using magnetic beads

(Miltenyi), and co-incubated with Teff cells for suppression assays. On day 4, cells were stained with anti-CD4 and eFluor 780 viability dye (Thermo Fisher). Divided $CD4^+$ Teff cells were determined by flow cytometry.

Basophil activation. The basophil activation test was performed using the Flow CAST Basophil Activation Test kit (Bühlmann Laboratories). 50 μl of whole blood from a healthy, non-allergic donor was incubated with 100 μl of patient or healthy control serum and 50 μl of buffer. Samples were incubated for 15 min at 37°C with staining antibodies for human PE anti-CCR3 (5E8, Biolegend) and FITC anti-CD63 (H5C6, BD Biosciences). Anti-IgE mAb (Southern Biotech) was used as a positive control for basophil activation.

Stimulation was stopped with the addition of lyse/fix for the lysis of red blood cells. Samples were then washed and assessed by flow cytometry. Basophils were identified as $SSC^{low}CCR3^+$ and activation was assessed by CD63 expression.

Generation of $Cblb^{H257L}$ mice. sgRNA design was facilitated through the CRISPOR design tool to minimize off-target effects. A guide was selected with predicted double-stranded cutting 8 nucleotides from the intended c.854A>T:p.H285L mutation (Synthego). A 121 bp repair template with the c.854A>T mutation as well as 3 synonymous change in the protospacer sequence were generated (PAGE Ultamer from Integrated DNA Technologies). Zygotes from C57BL/6 mice were microinjected with the sgRNA, repair template, and Cas9 protein (Integrated DNA Technologies). The resulting mice were screened by PCR and Sanger sequencing to select for mice bearing the p.H257L mutation. Mice were bred to generate mice homozygous for the p.H257L mutation. Homozygous $Cblb^{H257L}$ and WT C57BL/6 mice were bred and maintained under specific pathogen free conditions. Both female and male mice were used in all experiments.

Immunoblotting. Cells were lysed in 1% Triton buffer (150 mM NaCl, 25 mM Tris-Cl, pH 7.5, 5 mM EDTA) containing complete protease inhibitors (Roche). Cell lysates were separated by SDS-PAGE, transferred to nitrocellulose. The following antibodies were used for

immunoblotting - N-terminal directed CBL-B antibody (G-1, Santa Cruz Biotechnology), pY (4G10, Millipore), and C-terminal directed CBL-B antibody (D3C12), STAT3 (79D7), GAPDH (14C10), pY^{220} LAT (3584), pY^{526} SYK (C87C1), pY^{352}SYK (2701), pPLCγ1 (D6M9), and pPLCγ2 (3871) from Cell Signaling Technology. Protein band intensities were quantified by densitometry using the ImageJ software (Wayne Rasband, NIH).

Flow cytometry. Single cell suspensions of mouse spleens and lymph nodes were lysed using ACK lysis buffer (Life Technologies). Cell suspensions were incubated with TruStain FcX (anti-CD19/CD32, Biolegend) and eF506 viability (ThermoFisher) to exclude dead cells. For staining of surface molecules, cells were incubated on ice with fluorochrome conjugated mAbs against - CD3 (145-2C11), CD4 (GK1.5), CD8 (53-6.7), CD25 (PC61), CD69 (H1.2F3), CD62L (Mel-14), CD23 (B3B4), IgM (RMM-1), IgD (11-26c.2a), CD93 (AA4.1), B220 (RA3-6B2), CD21 (7E9), PD1 (RMP1-30), CXCR5 (L138D7), GL-7, CD117 (2B8), and FcεRI (MAR-1) from Biolegend, and FAS (15A7) from Thermo Fisher. For staining of transcription factors, cells were fixed and permeabilized using the eBioscience Transcription Factor Staining Buffer Set (Thermo Fisher) according to the manufacturer's directions. An antibody against Foxp3 (FJK-16s, Thermo Fisher) was used to identify Treg cells.

Human plasma cytokines and chemokines were measured using the BD CBA human inflammatory cytokine and chemokine kits (BD Biosciences) according to the manufacturer's directions.

Mouse splenocytes were loaded with Fluo-4 (Thermo Fisher) according to the manufacturer's directions. Cells were stained with antibodies against CD4 and B220. T cells were stimulated by the addition of 10 μg/ml anti-CD3 (2C11, Biolegend) and cross-linking with anti-Armenian Hamster IgG (Jackson Immunoresearch). 1 μM Ionomycin (Sigma-Aldrich) was added after 4 minutes.

Mouse splenocytes were stimulated by anti-CD3 cross-linking as described above. Cells were fixed and permeabilized using BD Cytofix Fixation buffer and BD Phosflow Perm buffer III

(BD Biosciences) according to the manufacturer's directions. Cells were stained with antibodies against CD4, B220, pS^{473} Akt (560343, BD Biosciences), and pT^{202}/Y^{204} Erk1/2 (13148, Cell Signaling).

All flow cytometry data was collected using an LSR Fortessa cell analyzer (BD Biosciences) and analyzed with FlowJo software (Tree Star).

Autoantibody detection. Human plasma IgG autoantibodies were measured using an autoantigen proteomic array (128 antigen panel) through the UT Southwestern Medical Center Microarray Core as previously described [14]. Autoantigens with a signal-to-noise ratio of >3 were analyzed. Heat maps are shown for antigens with a calculated p value of <0.05 by Student's t test. Autoantigen values were normalized to the geometric mean for all samples and visualized as a heat map using GraphPad PRISM.

Mouse sera was diluted in PBS and applied to slides of HEp-2 cells (Fisher) for 30 min. Slides were washed with PBS+1% Tween and bound IgG was detected with anti-mouse IgG conjugated to AlexaFluor 488 (Biolegend). Samples were scored by 3 blinded individuals.

*Mouse lymphocyte proliferation***.** Total B cells and $CD4^+CD25^-$ Teff cells were purified from mouse spleens using magnetic beads (Miltenyi Biotec). Cells were labeled with Cell Trace Violet (Thermo Fisher). B cells were plated at 200,000 cells per well in a 96 well plate with 100 ng/ml-10 µg/ml anti-IgM (Jackson ImmunoResearch). $CD4^+CD25^-$ Teff cells were plated at 200,000 cells per well in a 96 well plate coated with anti-CD3 (2C11, Biolegend). Teff cell proliferation was also examined in response to 1 µg/ml anti-CD3 and 10 µg/ml anti-CD28 (37.51, Biolegend).

On day 4, cells were stained with anti-CD4 or anti-B220 and eFluor 780 viability dye (Thermo Fisher). Divided $CD4^+$ T and B cells were determined by flow cytometry. Supernatants from $CD4^+CD25^-$ T cell cultures were collected, and IL-2 content was measured by ELISA (Thermo Fisher).

*Mouse Treg cell suppression assays***.** $CD4^+$ T cells were enriched for by negative selection using the $CD4^+$ T cell isolation kit (Miltenyi Biotec). $CD4^+CD25^-CD39^-$ Teff cells and

$CD4^+CD25^+CD39^+$ Treg cells were sorted using an MA900 FACS (SONY). Teff cells were labeled with Cell Trace Violet (Life Technologies) to monitor cell divisions. Teff cells were stimulated with 1 μg/ml anti-CD3 mAb (2C11, Affymetrix) in the presence of T cell depleted splenocytes treated with 25 μg/ml mitomycin C (Santa Cruz Biotechnology) in the presence or absence of Treg cells. On day 4, Teff cell divisions were determined by flow cytometry.

Immunizations. Mice were immunized *i.p.* with 10 μg TNP-Ficoll (F-1300, Biosearch) or 10 μg TNP-LPS (T-5065, Biosearch). Sera was collected on days 0 and 14. Mice were immunized either *i.p.* or in the bilateral hocks with 10 μg of TNP-KLH (T-5060, Biosearch) in alhydrogel adjuvant (vac-alu-250, Invivogen), and boosted at day 14 with 2.5 μg of TNP-KLH in alum. Sera were collected at days 0 and 21. To examine GC B cell and Tfh cell generation, the draining LNs (inguinal and popliteal) were harvested on day 10 from hock immunized mice.

ELISAs. For detection of total serum immunoglobulins, 96-well plates were coated with anti-IgG, IgM, and IgA from Southern Biotech. After serum incubation, alkaline phosphatase-conjugated rat anti-mouse IgG, IgM, or IgA (Southern Biotech) were applied to plate for 2 h at room temperature. Bound secondary antibodies were detected by incubation with p-nitrophenyl phosphate (PNPP) substrate (Sigma-Aldrich). Serum concentrations of MCPT-1 were determined by ELISA per the manufacturer's directions (Invitrogen).

LAMP-1 mobilization on BMMCs. BMMCs were cultured from bone marrow collected from 8-10-week-old mice as detailed in reference [26]. BMMCs were incubated with 50 ng/ml of anti-DNP IgE (clone SPE-7, Sigma-Aldrich). After 24 h, BMMCs were stimulated with 0-25,000 ng/ml DNP-BSA (Sigma Aldrich) and stained for surface LAMP-1 (clone 1D4B, Biolegend).

Passive oral anaphylaxis. Mice were passively sensitized 10 μg anti-trinitrophenyl (TNP)-IgE *i.p.*, a gift from Dr. Fred Finkelman, and injected with an implantable temperature transponder (IPTT-300, Bio Medic Data Systems). The following day, mice were challenged by oral gavage of 2.5 mg TNP-BSA (Biosearch, T-5050). Core body temperatures were measured serially for 60

min after gavage using a DAS-6001 Smart Probe (Bio Medic Data Systems). Blood was collected for MCPT-1 measurement at 60 min after challenge.

SUPPLEMENTAL REFERENCES

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SUPPLEMENTAL LEGEND

Supplemental Figure 1. Increased autoantibodies in P1 and P2. A heatmap of reactivity of IgG antibodies against self-antigens in 16 HCs, P1, and P2. Only the 19 autoantigens for which binding was significantly higher in the patients ($p<0.05$) are shown. Binding was normalized to the geometric mean for each antigen.

Supplemental Table 1. Patient laboratory values.

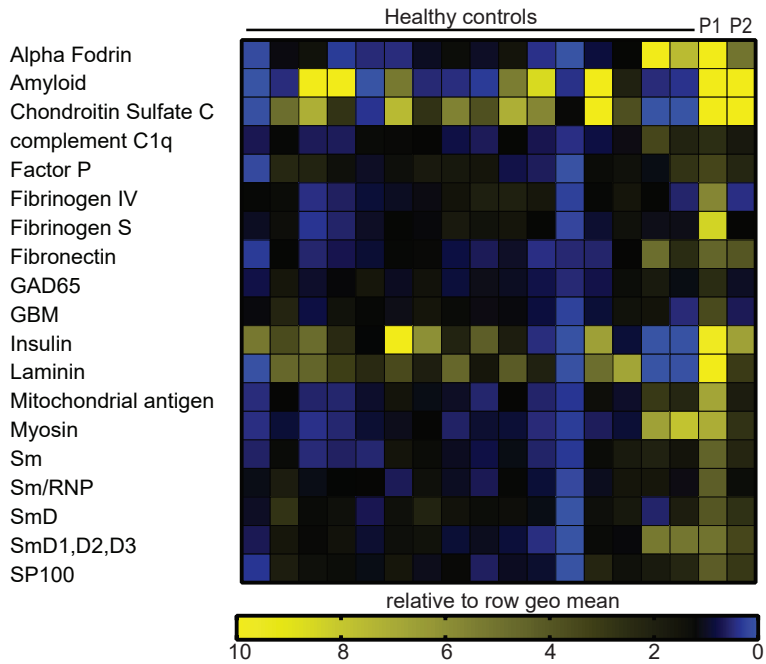
	Patient 1	Patient 2	Patient 3
***CBLB* mutation**	c.854A>T (p.His285Leu)	c.1486C>T (p.Arg496X)	c.1392C>G (p.Cys464Trp)
Age at study (years)	16	11	4
Blood Counts			
Hemoglobin (g/L)	9.6	9.3	***6.8***
Platelets (cells/μL)	280,000	190,000	***37,000***
WBCs (cells/μL)	8,800	3,700	6,300
Neutrophils (cells/μL)	6,700	1,300	1,300
Lymphocytes (cells/μL)	1,600	2,200	4,200
Lymphocyte subsets (cells/μL)*			
$CD3^+$	1,420	2,060	1,880
$CD3^+CD4^+$ cells	843	1,329	850
$CD3^+CD8^+$ cells	597	736	700
$CD19^+$ cells	455	404	1,010
$CD3^-CD56^+$	363	23	13
Immunoglobulins (mg/dL) [normal range for age] (26, 27)			
IgG	***199*** [639-1349]	903 [639-1349]	560 [424-1051]
IgA	***<23*** [70-312]	126 [70-312]	30 [14-123]
IgM	***33.6*** [56-352]	66 [56-352]	***38*** [48-168]
IgE (IU/ml)	<2 [<537]	39.5 [<696]	38 [<307]
Vaccine titers			
	Tetanus IgG <0.1 IU/ml Diphtheria IgG <0.01 IU/ml Pneumococcal <0.1 μg/ml (for all strains tested)	Tetanus IgG 0.43 IU/ml N/A N/A N/A	Tetanus 0.16 IU/ml Diphtheria 0.3 IU/ml ***Pneumococcal <17 U/ml (for all strains tested)***
Basophil activation [data for 5 HCs]			
[HCs 1.18±0.6% $CD63^+$ basophils]	***30.8***	0.38	3.33
Serum Cytokines (pg/ml) and chemokines [data for 3 HCs]			
IL-6 [6.3±1.1]	***7,254***	***13***	***11,321***
TNF-α [8.0±0.7]	***21***	***49***	***1,093***
IL-10 [6.4±1.3]	***68***	***23***	***24***
CXCL9 [38.7±25.9]	***674***	***81***	***314***
CXCL10 [1,313±683]	***22,204***	1,432	***5,562***

***Lymphocyte subsets determined on a different day than the CBC. Abnormal values are italicized and in bold.

Supplemental Table 2. Rare homozygous coding variants identified in patients

Case	Chr	start (genomic coordinate)	gene	Reference seq: nucleotide change	Reference seq.: protein change	variant_type	dbSNP	OMIM	phyloP	PopFreqMax	CADD_RAW
P1	chr1	114515923	HIPK1	NM_198268.2:c.3422A>C	NM_198268.2:p.His1141Pro	Missense		608003	7.078	N/A	3.97531
	chr1	115430258	SYCP1	NM_001282541.1:c.1202A>T	NM_001282541.1:p.Asn401Ile	Missense		602162	0.457	N/A	0.640877
	chr3	46491431	LTF	NM_002343.5:c.970A>T	NM_002343.5:p.Ile324Phe	Missense	rs138966671	150210	-3.276	0.022	-1.35074
	chr3	105464836	CBLB	NM_001321786.1:c.8544>T	NM_001321786.1:p.His2851Leu	Missense		604491	7.942	N/A	6.80295
	chr5	76171302	S100Z	NM_130772.3:c.118C>G	NM_130772.3:p.Arg40Gly	Missense	rs148288662	610103	2.299	0.0031	2.50139
	chr15	90784558	GDPGP1	NM_001013657.2:c.418C>T	NM_001013657.2:p.Arg140Trp	Missense	rs147221532		0.711	0.011	5.83929
	chr15	91810848	SV2B	NM_001323031.1:c.1183G>A	NM_001323031.1:p.Val395Met	Missense	rs144134786	185861	2.973	0.0003	4.38217
	chr18	44069093	LOXHD1	NM_144612.6:c.5705G>T	NM_144612.6:p.Gly1902Val	Missense		613072 [Deafness, autosomal recessive 77]	5.948	N/A	6.40097
	chr20	47633816	ARFGEF2	NM_006420.2:c.4346A>C	NM_006420.2:p.Asn1449Thr	Missense	rs200473895	605371 [Periventricular heterotopia with microcephaly]	9.325	0.0011	5.5907
	chr8	144911455	PLEKHD	NM_078480.2:c.19G>T	NM_078480.2:p.Ala7Ser	Missense	rs782322841	604819 [Verheij syndrome]	1.819	0.0014	4.36049
P2	chr1	168250389	TBX19	NM_005149.2:c.61A>G	NM_005149.2:p.Asn21Asp	Missense	rs372107667	604614 [Adrenocorticotropic hormone deficiency]	1.561	0.0022	-0.173439
	chr1	197061161	ASPM	NM_018136.4:c.9320G>A	NM_018136.4:p.Arg3107Gln	Missense	rs587783290	605481 [Microcephaly 5, primary, autosomal recessive]	1.054	N/A	3.99942
	chr2	26408071	GAREM2	NM_001168241.1:c.1354T>A	NM_001168241.1:p.Ser452Thr	Missense	rs758078493	617999	2.095	0.0001	1.19151
	chr2	26967810	DRC1	NM_145038.2:c.1390C>T	NM_145038.2:p.Arg464Cys	Missense	rs377330459	615288 [Ciliary dyskinesia, primary, 21]	0.073	0.0003	3.54294
	chr2	26951083	KCNK3	NM_002246.2:c.832A>T	NM_002246.2:p.Ser278Cys	Missense	rs368524386	603220 [Pulmonary hypertension, primary, 4]	1.609	0.0006	0.220446
	chr2	176944985	EVX2	NM_001080458.1:c.1278_1286dupCGG	NM_001080458.1:p.Gly427dup	disruptive_inframe_insertion		142991		N/A	
	chr3	105438896	CBLB	NM_001321786.1:c.1486C>T	NM_001321786.1:p.Arg496*	Nonsense	rs535219619	604491	3.014	0.0008	11.2936
	chr3	130368083	COL6A6	NM_001102608.1:c.5410T>C	NM_001102608.1:p.Ser1804Pro	Missense	rs775840065	616613	-0.247	0	0.894609
	chr7	4014026	SDK1	NM_152744.3:c.1843G>A	NM_152744.3:p.Ala615Thr	Missense		607216	-0.032	N/A	-0.319415
	chr11	285263	NLRP6	NM_138329.2:c.2638G>A	NM_138329.2:p.Gly880Ser	Missense	rs147916706	609650	-1.885	0.0037	-1.12583
	chr11	1031874	MUC6	NM_005961.2:c.295G>A	NM_005961.2:p.Val99Met	Missense	rs199597580	158374	1.273	0.0013	1.14174
	chr19	52521356	ZNF614	NM_025040.3:c.143G>T	NM_025040.3:p.Gly48Val	Missense&splice_region_variant	rs868601619		0.732	N/A	4.69654
	chr22	32439303	SLC5A1	NM_000343.3:c.35C>T	NM_000343.3:p.Ala12Val	Missense	rs150288967	182380 [Glucose/galactose malabsorption]	1.107	0.0023	1.62467
P3	chr1	100949960	CDC14A	NM_033312.2:c.1090A>G	NM_033312.2:p.Ile364Val	Missense	rs140849467	603504 [Deafness, autosomal recessive 32, with or without immotile sperm]	4.376	0.0034	1.29271
	chr1	182025963	ZNF648	NM_001009992.1:c.1183C>T	NM_001009992.1:p.Pro395Ser	Missense			-0.13	N/A	-0.274642
	chr3	99865873	CMSS1	NM_032359.3:c.121C>G	NM_032359.3:p.Pro41Ala	Missense			-0.178	N/A	0.039348
	chr3	105438990	CBLB	NM_001321786.1:c.1502C>G	NM_001321786.1:p.Cys454Trp	Missense		604491	0.764	N/A	3.94929
	chr3	108100406	MYH15	NM_014981.1:c.5827G>T	NM_014981.1:p.Val1943Phe	Missense	rs201226192	609929	0.462	0.0061	3.18001
	chr3	122880229	PDIA5	NM_006810.3:c.1406C>T	NM_006810.3:p.Ala469Val	Missense		616942	6.558	N/A	7.33522
	chr4	1016024	FGFRL1	NM_001004356.2:c.113G>A	NM_001004356.2:p.Arg38Gln	Missense	rs557619683	605830	6.017	0.015	2.12777
	chr4	3318224	RGS12	NM_198229.2:c.327A>C	NM_198229.2:p.Glu109Asp	Missense	rs748359816	602512	-1.507	0.0001	0.17139
	chr5	109049221	MANZA1	NM_002372.3:c.136G>A	NM_002372.3:p.Gly46Ser	Missense	rs139275194	154582	4.087	0.0045	3.7314
	chr5	118485778	DMXL1	NM_001290321.2:c.4256_4263del5TTACTCinsT	NM_001290321.2:p.Cys1419_Ser1421delinsLeu	stop_gained&Missense&disruptive_inframe_deletion		605671		N/A	
	chr5	118728798	TNFAIP8	NM_001286814.1:c.355C>G	NM_001286814.1:p.His119Asp	Missense		612111	3.934	N/A	5.55333
	chr5	140773600	PCDHGA8	NM_032088.1:c.1220G>A	NM_032088.1:p.Arg407Lys	Missense		606295	-0.129	N/A	-0.433971
	chr5	141694117	SPRY4	NM_030964.3:c.626G>A	NM_030964.3:p.Cys209Tyr	Missense	rs148983803	607984 [Hypogonadotropic hypogonadism 17 with or without anosmia]	5.82	0.0082	5.55485
	chr6	43153786	CUL9,SRF	NM_015089.3:c.844G>A	NM_015089.3:p.Gly282Arg	Missense	rs148687309	607489	6.041	0.0023	5.06693
	chr6	150054587	SCAF8	NM_001286188.1:c.34_35delCT	frameshift_variant			616024		N/A	
	chr12	6143865	VWF	NM_000552.3:c.2674G>A	NM_000552.3:p.Val892Ile	Missense	rs201072235	613160 [von Willebrand disease]	2.849	0.001	5.41717
	chr12	8248229	NECAP1	NM_015509.3:c.709G>C	NM_015509.3:p.Val237Leu	Missense	rs752918460	611623 [Epileptic encephalopathy, early infantile, 21]	2.874	0.0045	1.44797
	chr12	11905438	ETV6	NM_001987.4:c.88T>C	NM_001987.4:p.Ser30Pro	Missense		600618 [Leukemia, acute myeloid, somatic, Thrombocytopenia 5]	4.159	N/A	2.38149
	chr12	66698889	HELB	NM_033647.4:c.566T>C	NM_033647.4:p.Leu189Ser	Missense	rs113658249	614539	-0.157	0.0053	-1.51899
	chr12	66704259	HELB	NM_033647.4:c.1551G>A	NM_033647.4:p.Trp517*	Nonsense	rs148603418	614539	6.068	0.0002	11.3078
	chr13	113826267	PROZ	NM_001256134.1:c.1117T>C	NM_001256134.1:p.Trp373Arg	Missense		176895 [Protein Z deficiency]	1.867	N/A	2.16364
	chr14	23057936	DAD1	NM_001344.3:c.128G>C	NM_001344.3:p.Gly43Ala	Missense		600243	4.888	N/A	3.02155
	chr15	65499287	CILP	NM_003613.3:c.257G>A	NM_003613.3:p.Cys86Tyr	Missense	rs773568646	603489 [Lumbar disc disease, susceptibility to]	7.744	0.0001	6.44717
	chr16	20975614	DNAH8	NM_017539.2:c.9592C>T	NM_017539.2:p.Pro3198Ser	Missense	rs755055680	603334	10.003	0	4.24907
	chr17	79966910	ASPSCR1	NM_001251888.1:c.951_965delCGTGGACCGGGAGCC	NM_001251888.1:p.Val318_Pro322del	disruptive_inframe_deletion	rs372283666	606236 [Alveolar soft-part sarcoma]		0.025	
	chr18	12706682	PSMG2	NM_020232.4:c.191C>T	NM_020232.4:p.Thr64Ile	Missense	rs115693908	609702	2.187	0.011	0.359226
	chr20	307296	SOX12	NM_006943.3:c.728A>G	NM_006943.3:p.Glu243Gly	Missense	rs73574574	601947	2.367	0.021	2.01524
	chr20	2739620	EBF4	NM_001110514.1:c.1792T>A	NM_001110514.1:p.Ser598Thr	Missense	rs146931761	609935	4.874	0.012	1.91883
	chrX	2778018	GYG2	NM_003918.2:c.842A>G	NM_003918.2:p.His281Arg	Missense		300198	1.727	N/A	-0.755099
	chrX	22095767	PHEX	NM_000444.4:c.610A>T	NM_000444.4:p.Ile204Phe	Missense		300550 [Hypophosphatemic rickets, X-linked dominant]	4.238	N/A	5.46451
	chrX	48399738	TBC1D25	NM_020536.2:c.141G>C	NM_020536.2:p.Leu47Phe	Missense		311240	3.069	N/A	1.27505
	chrX	136649132	ZIC3	NM_003413.3:c.282T>A	NM_003413.3:p.His94Gln	Missense		300265 [Congenital heart defects, nonsyndromic, 1, X-linked]	-0.236	N/A	-0.64845

PopMaxFreq (Population maximum frequency): indicates the highest frequency of the variant observed in databases. PhyloP scores indicate evolutionary conserved positions (high positive). CADD (Combined Annotation-Dependent Depletion) ranks genetic variants, including single nucleotide variants (SNVs) and short inserts and deletions (InDels), throughout the human genome reference assembly. CADD_RAW score above 4 are considered most as likely pathogenic (33)



Supplemental Figure 1