

Supplemental Data

Mutations in *EXTL3* Cause

Neuro-immuno-skeletal Dysplasia Syndrome

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Supplemental Note: Case Reports

Case report family A

Individual II-1 from family A was born at 37 weeks with an APGAR score of 9/9. Shortly after birth, she developed tachypnea with an increasing need for oxygen. At 20 weeks gestation a fetal ultrasound showed skeletal abnormalities, kidney and liver cysts. After birth, the skeletal abnormalities included short limbs, platyspondyly and brachydactyly (Figure 1). The girl had horseshoe-shaped kidneys with hyperechoic small-sized cysts and progressive renal insufficiency. Her liver also contained some moderately sized cysts, but liver function was normal. Brain abnormalities were detected by MRI including a hypoplastic anterior part of the corpus callosum, ventriculomegaly, and several small parenchymal hemorrhages. Erythrocytes, white blood cell counts and platelet number were normal. Because of cytomegalovirus (CMV) pneumonitis, her white blood cells were subtyped. Upon immunophenotyping, the child was found to have no circulating T cells, whereas B and NK cell numbers were normal (Figure 4A). The girl died at age 7 weeks due to renal insufficiency.

Case report family B

The first child of family B (B:II-1, Figure 1, Table S1) was born at term after an uncomplicated pregnancy with low birth weight and birth length but normal occipitofrontal head circumference (OFC) and with contractures in both elbows. He developed seizures in the neonatal period and West syndrome at age of six months which both responded well to anticonvulsive therapy. Severe global developmental delay and microcephaly became evident at age of 6 months. The further course of disease was characterized by very slow development, severe muscular hypertonia and progressive microcephaly. Extensive clinical and metabolic work up gave normal results except a mildly elevated heparan sulfate concentration in plasma. The X-ray at age of 23

months displayed a delayed ossification and features of spondyloepiphyseal dysplasia (detailed description in Table S1). At the last examination the 8 years and 3 months old boy was affected by profound global developmental delay, stereotypic movements, severe muscular truncal hypotonia, and hypertonia in limbs. He was not able to walk, sit, crawl, speak or to get in social interaction. He was overweight (+2.1SD) with a disproportionate short stature (-2.0SD) but surprisingly normal OFC (+1.0SD). Facial dysmorphism included coarse facial features, long face, deep set eyes, long nose, bulbous nasal tip, long un-modulated philtrum, full cheeks, open mouth appearance and salivation. His neck was short, his chest broad. He had short upper and lower limbs, dimples and contractures of both elbows. His hands and feet were short and puffy, he had brachydactyly and clinodactyly of digit V. MRI of the brain, ECG, EEG, ophthalmological examination as well as extensive laboratory and metabolic analyses gave normal results, ultrasound of the abdomen revealed increased echogenicity of liver. X-ray revealed deceleration of bone age and the previously described features of spondyloepiphyseal dysplasia (Figure 1). SNP-array was done with unrevealing results.

The boy has experienced frequent viral upper airway infections and two pneumonias that required admission. He displayed moderate T cell lymphopenia affecting both CD4+ and CD8+ T cells, while B and NK cell count were normal. Immunoglobulins were moderately decreased, with proportionate reduction of IgG subclasses. Response to the vaccines was generally adequate, however no response to a pertussis vaccine could be detected. He did not display any features of autoimmunity.

The second child of family B (B:II-2), a girl, was born at term after an uncomplicated pregnancy with normal measure. Microstomia, preaxial polydactyly of the left hand and postaxial polydactyly on right foot were noticed directly after birth. Polydactyly was interpreted to be familial as the mother and several other maternal family members were similarly affected. At the age of three weeks microcephaly, massive eosinophilia in the blood cell differentiation and

erythrodermia with scaling of skin was observed. At three months of age, an analysis of lymphocyte subsets revealed absent CD8⁺ T-cells with abundant CD4⁺ T-cells, normal B cell count and elevated NK cell count. Whether the CD4⁺ T cells were maternal was not tested. Eosinophils and IgE were elevated. The diagnosis of SCID with skin involvement was established. ZAP-70 gene analysis gave inconspicuous results. IgG and IgM were above the normal range before IVIG administration. An allogeneic stem cell transplantation from a matched unrelated donor following conditioning with treosulfan, fludarabine and alemtuzumab was performed at the age of 20 weeks. Engraftment was prompt and donor chimerism remains >80% with >90% in T cells two years after HSCT. A transient GvHD I° of the skin occurred, without any further severe transplant related complications.

In addition to the immunological features severe developmental delay, truncal muscular hypotonia and hypertonia in limbs with contractures in elbows were evident at age of 12 weeks. At the age of 20 weeks seizures and nystagmus occurred. At 1 year and 5 months the girl was affected by severe developmental delay and generalized muscular hypotonia. She was unable to walk, sit, crawl, speak or to get in social interaction. Facial dysmorphism consisted in microcephaly (OFC -3,3SD), round face, up-slanting palpebral fissures, prominent nose with broad nasal tip, full cheeks, and small mouth. In addition, she had a short neck, dimples and contractures of both elbows, small and puffy hands, brachydactyly, hexadactyly on the left hand, hexadactyly on the right foot and generalized hypertrichiosis.

MRI of the brain at four months of life revealed multiple kinking of the ACI, but the MRI at 1 year and 11 months was normal. Echocardiography at five months showed hypersarrhythmia. ECG at five months discovered a small ASD with left/right shunt, aneurysm of atrial septum and discrete dilatation of left atrium, which have been resolved at 1 year and 11 months. X-Ray displayed dissociated skeletal age and features of spondyloepiphyseal dysplasia (Figure 1A). Ultrasound

of abdomen showed a prominent uterus but normal liver. Most recently measured immunological parameters were normal.

Case report family C

The affected individual in family C was born after uncomplicated pregnancy at 40 weeks gestation. She developed hyperbilirubinemia and transient hypoglycemia which resolved. She was evaluated in the neonatal period because of facial dysmorphism like full lips, large tongue, bifid uvula and coarse facial features and significant lumbar gibbus deformity. She was tested for storage disorder but repeated measurements of urine mucopolysaccharides and oligosaccharides were negative. Additional testing included a g-banded Karyotype, transferrin isoelectric focusing to rule out carbohydrate deficient glycoprotein syndrome and full metabolic work-up including plasma amino acids, urine organic acids and acylcarnitine profile and targeted sequencing of the *FGFR3* gene, all of which were normal. She had an echocardiogram at 2 years of age that showed a patent foramen ovale (PFO) and a cleft of the anterior leaflet of the mitral valve. Renal ultrasound was normal. Because of ongoing concern for a storage disorder lysosomal enzyme activity testing was done in fibroblasts and in white blood cells and were normal. She has a history of repeated upper airway infections particularly in the first 5 years of life. Complete blood counts have shown fluctuating numbers of white blood cells with normal neutrophil numbers but multiple instances of mild lymphopenia (lowest value 0.45×10^9 lymphocytes/L, normal values between 1.5 and 7×10^9 lymphocytes/L). At 1 year of age she underwent decompression at the level of the foramen magnum due to craniocervical stenosis. She required bracing from age 4 years until last evaluation at 12 when she underwent posterior spinal fusion with decompression, followed by anterior spinal fusion with tibial strut graft.

Case report family D

Individual IV-1 from family D presented prenatally with omphalocele detected at the 12 weeks gestation ultrasound and with hepatic cysts and right lung sequestration suspicion at 26 weeks. She was born at 37 weeks (elective cesarian section) with an APGAR score of 9/10 and birth weight of 3210g. Postnatal period was complicated by neonatal hyperbilirubinemia and surgery of omphalocele at the second day of life. She had muscular hypotonia and mild motor development delay, and normal cognition with presently mild learning difficulties. Her stature was short and due to failure to thrive she was fed by nasogastric tube for several months. She developed a spondyloepimetaphyseal dysplasia characterized by cervical instability associated with C1 malformation and spinal cord compression which required surgical intervention at 2 years of age; severe platyspondyly and vertebral body anomalies leading to progressive kyphoscoliosis and spinal surgery was required: T3-L4 arthrodesis at 11 years of age; pectus excavatum and rib deformity; coxa valga; small upper femoral epiphyses; and radial deformity with head subluxation; and progressive deformity of the pelvis. At last examination at 12 years of age she had coarse facial features, strabismus and excess skin in palmar and plantar regions. The hepatic follow up revealed 7 non progressive liver cysts, normal liver function, and the hypothesis of biliary hamartomas was raised. She required cardiac follow-up due to interauricular communication with interauricular septum aneurysm (surgically corrected) and moderate mitral insufficiency.

Enzymatic studies excluded gangliosidoses GM1 and GM2 and mucopolysaccharidoses IVA, VI and VII. Urinary oligosaccharides and mucopolysaccharides electrophoresis was normal and total urine glycosaminoglycan measurements at 6 months of age was 15 mg/mmol creatinine (reference value is 11-35).

Individual III-1 from family D, a cousin of individual D:IV-1, was born at term after an uneventful pregnancy. Skeletal and craniofacial anomalies were detected from the neonatal period on. They

evolved to a spondyloepimetaphyseal dysplasia, characterized by cervical spine malformation with instability and odontoide peg malformation; severe platyspondyly and severe kyphoscoliosis; pectus excavatum; radial deformity; progressive deformity of the pelvis, coxa valga and flat epiphyses at the upper femora and to a lesser extent the knees; brachydactyly bilateral second metacarpals; pseudoepiphyses. Kyphosis required repeated surgical interventions at the age of 10 years. The boy had borderline level of cognition. Several metabolic studies were performed and gave normal results: enzymatic studies excluded gangliosidoses GM1 and GM2 and mucopolysaccharidoses IVA, VI and VII; urinary oligosaccharides and mucopolysaccharides electrophoresis; total urine glycosaminoglycan measurements at 10 years of age was 12 mg/mmol creatinine (reference value is 4-11). He was lost for follow-up after 22 years of age and died at the age of 30 years of unknown reason.

For individual III-2 from family D, a cousin of individual D:III-1 and D:IV-1 were only limited information available. She is a 38 year-old female with similar skeletal features than the other two cousins but apparently less severe. She had a normal neurodevelopment. Several metabolic studies were performed and gave normal results: very long chain fatty acids, urine glycosaminoglycan measurements: 2 mg/mmol creatinine (reference value if 1-5).

Case report family E

Individual E:II-1 was the first child of non consanguineous parents of Indian origin. Born at 38 weeks gestation, he was noted to be dysmorphic with truncal hypotonia but a specific diagnosis was not made. He suffered from omphalitis caused by *Staphylococcus aureus* at eight days of age. Following this he developed a widespread eczematoid rash which improved with topical corticosteroids. He suffered some self limiting episodes of cyanosis. At two months of age he had a respiratory arrest associated with septicemia caused by *S. aureus* requiring ventilation

and inotropic support on the intensive care unit. He was found to have small dysplastic kidneys with impaired renal function and an immunodeficiency was suspected. Immunological testing showed low T cell count with an oligoclonal pattern of V beta families and an absence of naïve T cells and TRECs. Natural killer and B cell numbers were normal. His skin rash was considered to be Omenn syndrome. By this stage he had multiple joint contractures in limbs, he demonstrated abnormal limb movements and myoclonic jerks interpreted as seizures. He was also found to have hypothyroidism with an elevated TSH. In the further course of disease he suffered from frequent episodes of apnoea probably associated with poor clearance of secretions. He required several admissions to intensive care for ventilator support became oxygen dependent. He showed very poor development and head growth. In conjunction with the family it was considered inappropriate to perform a haemopoietic stem cell transplant (HSCT) given the severity of the non-immunological problems. He died at 6 months of age from respiratory failure.

Individual E:II-2, the brother of individual E:II-1. Born was born at 37 weeks gestation with a birthweight of 2.520 kg and an Apgar score of 6/10. He was noted to have very similar dysmorphic features to his brother at birth as well as truncal hypotonia. The size of his kidneys was within the normal range and though echo bright they were not considered to be significantly dysplastic on ultrasound examination. Early immunology testing confirmed an absence of T cells with normal B and NK counts resulting in an immunoglobulin treatment and antibiotic prophylaxis. He suffered from recurrent apnoeas and poor secretion clearance and developed an oxygen dependence. He showed very poor development, he suffered from seizures and had an evidence of a bulbar palsy. At 6 months he developed an Omenn type skin rash associated with the appearance of circulating T cells with a memory phenotype and a restricted oligoclonal repertoire. As in his brother's case, HSCT was considered inappropriate. He died at 10 months of age of respiratory failure.

Supplemental Figures



Figure S1. Mild dysmorphic facial features in *EXTL3* affected individuals. The nine affected individuals displayed mild dysmorphic facial features, including a coarse face, full cheeks, depressed nasal bridge, and a broad nasal tip. For affected individuals A:II-1 and D:III-2 there is no permission to publish photographs, and for affected individuals D:III-1, E:II-1 and E:II-2 no photographs were available.

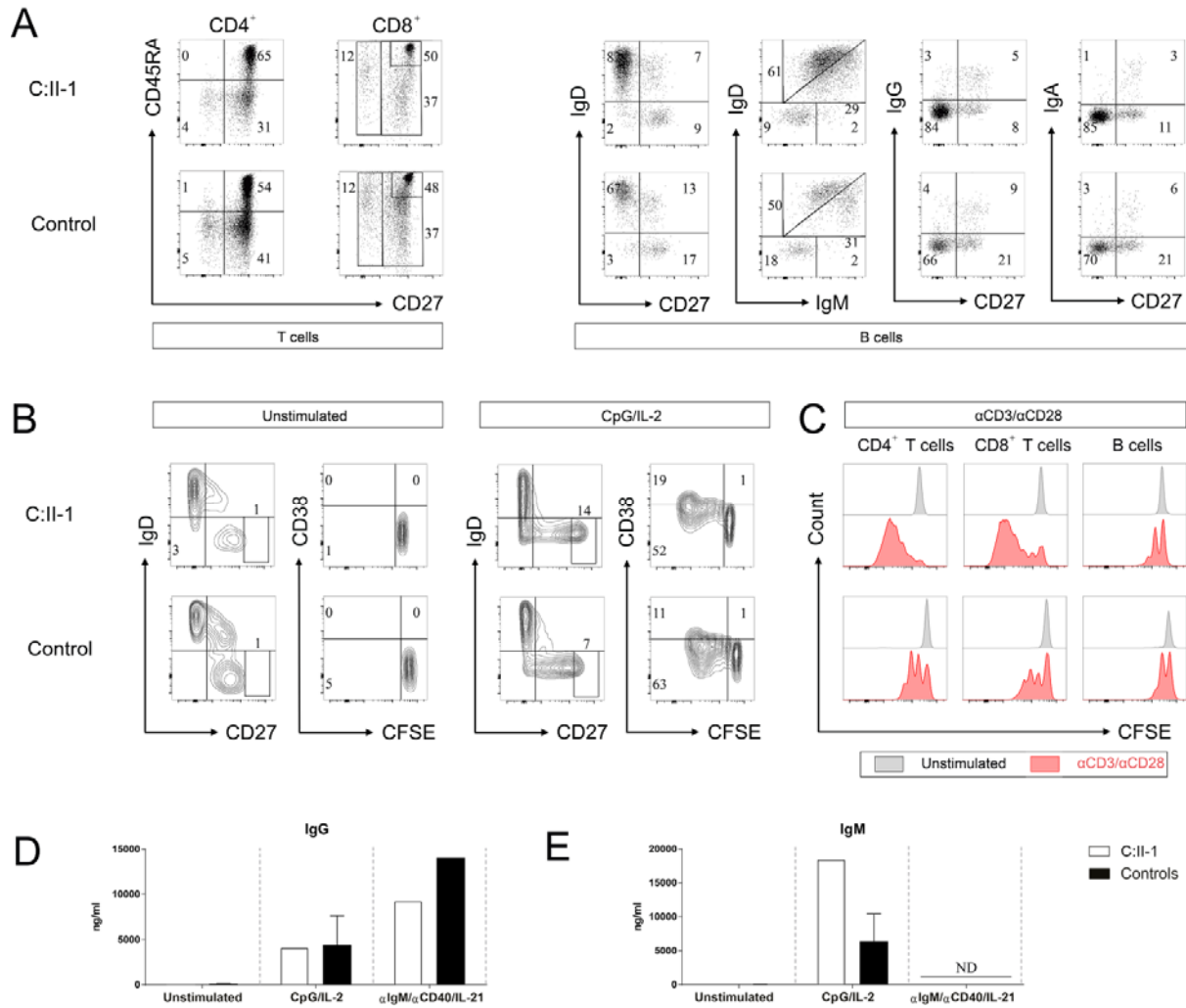


Figure S2. Normal B and T cell phenotype in affected individual C:II-1. Data are shown for affected individual C:II-1 and a healthy parent as the control. Same gating strategy used as described for Figure 4. (A) T and B cell subsets. (B) CFSE-labeled PBMCs were cultured with medium or CpG/IL-2. (C) CFSE-labeled PBMCs were cultured with either medium or anti-CD3/anti-CD28. (D, E) Levels of IgG and IgM in the supernatants after 6 days of culture with either medium, CpG/IL-2 or anti-IgM/anti-CD40/IL-21, ND not determined. Error bars indicate SEM (N=3).

	Family A	Family B	Family B	Family C	Family D	Family D	Family D	Family E	Family E
Individual	II-1	II-1	II-2	II-1	III-1	III-2	IV-1	II-1	II-2
Gender	female	male	female	female	male	female	female	male	male
Age at last examination	7 weeks	8 years	1.5 years	12 years	24 years	25 years	12 years	5 months	8 months
Ethnicity	Turkish	Turkish	Turkish	Colombian (Hispanic)	Portuguese	Portuguese	Portuguese	Indian	Indian
Consanguinity of parents	yes	yes	yes	possibly	possibly	possibly	possibly	no	no
Outcome	deceased at 7 wks	alive	alive	alive	deceased at 30 yrs	alive	alive	deceased at 6 mo	deceased at 10 mo
Measurements									
Weight (kg/SD)	2.3/ <2.5	43.3/ +2.1	9.53/ -1.64	41/ -0.1	26 at 10 yrs	n.d.	24.6 at 11 yrs	3.5 at 15 wks	6.14 at 4 mo
Length (cm/SD)	42/ <2.5	120/ -2.0	80/ -1.67	141/ -1	125/ -2 at 10 yrs	n.d.	109 at 11 yrs	nd at 15 wks	n.d.
Occipitofrontal circumference (OFC) (cm/SD)	33/ -2.0	54.3/ +1.0	44/ -3.73	54/ +1	55/ >2 at 10 yrs	n.d.		35.5 at 15 wks	42.6 at 7 mo
Neurological findings									
Intellectual disability	n.d.	severe	severe	mild	normal/borderline cognition	no	no (learning difficulties)	n.d.	n.d.
Seizures	n.d.	+	+	no	n.d.	n.d.	no	+	+
Dysmorphism/ anomalies	coarse face, hypertelorism, single palmar crease	coarse face, broad nasal tip, single palmar crease	coarse face, broad nasal tip, single palmar crease	coarse face, full cheeks, significant thoraco-lumbar gibbus	n.d.	n.d.	coarse face, broad nasal tip, single palmar crease	coarse face, hypertelorism, prominent nose, abnormal palmar crease	single palmar crease
Skeletal findings									
	short stature	disproportionate short stature, short limbs		disproportion with normal stature and short trunk	disproportionate short stature	disproportionate short stature, milder than cousins	disproportionate short stature	osteopenia with hairline fractures of tibia and femur	Normal for age (<1 month old)
Long bones	metaphyseal abnormalities	dislocated and dysplastic radial head, small capital femoral epiphyses	delayed ossification of radial epiphysis and femoral epiphysis	coxa valga with small femoral heads and small greater trochanteric epiphyses	radial head dislocation, progressive deformity, flat epyphyses	radial head luxation, progressive deformity, flat epiphyses	dislocated and dysplastic radial head, small capital femoral epiphyses	metaphyseal dysplasia	not significant
Hands/feet	brachydactyly, absent V middle phalanx and delayed carpal bone ossification	delayed ossification and short carpal bones, short middle and distal phalanx and cone-shaped epiphyses at middle phalanx II-IV, brachydactyly, clinodactyly	delayed carpal bone ossification, shorter middle and distal phalanges, brachydactyly, clinodactyly, polydactyly (familial)	short and prominent tufts of the distal phalanges in the medio-lateral dimension	mild brachydactyly, 2nd metacarpal pseudoepphyysis, delayed carpal bone age	short middle phalanx V	mild brachydactyly, delayed carpal bone ossification	absent V middle phalanx ossification	short 1st metacarpal and phalanges, absent V middle phalanx ossification
Spine and ribs	severe platyspondyly	possible mild platyspondyly	possible mild platyspondyly	severe platyspondyly, dextroconvex scoliosis of cervical spine, thoracolumbar kyphosis, mild pectus excavatum, with bulbous anterior rib ends	severe platyspondyly, complex cervical malformation, pectus excavatum with rib deformity	severe platyspondyly	severe platyspondyly, cervical malformation, pectus excavatum with rib deformity	severe platyspondyly	severe platyspondyly
Pelvis	n.d.	sloping acetabular roof with shallow lateral notches, coxa valga, small capital femoral epiphyses	n.d.	n.d.	sloping acetabular roof with shallow lateral notches, coxa valga, small capital femoral epiphyses	n.d.	n.d.	sloping acetabular roof with shallow lateral notches, coxa valga, small capital femoral epiphyses	n.d.
Immunological findings									
T cells	T-neg SCID	T-neg SCID	T-neg SCID	normal	n.d.	n.d.	n.d.	Omenn picture with confirmed oligoclonal T cell expansions	absent T cells, from 5-6 months oligoclonal T cell expansions with mild
B cells	normal	normal	normal	normal	n.d.	n.d.	n.d.	normal	normal
Leukopenia	-	+	-	several instances of lymphopenia	-	-	-	-	-
Eosinophilia	-	-	+	-	-	-	-	+	+
Allogeneic HSCT	-	-	+ at 20 wk of age	-	-	-	-	-	-
Other findings									
Heart abnormalities	-	-	not significant	not significant	-	-	moderate mitral insufficiency	-	-
Other	liver cysts, horseshoe kidney		perivascular calcification in basal ganglia		paraplegia - lesion at spinal surgery		liver cysts, omphalocele	no corticomedullary distinction in kidneys	
Homozygous mutation	EXTL3 c.1537C>T; p.R513C	EXTL3 c.2008T>G; p.Y670D	EXTL3 c.2008T>G; p.Y670D	EXTL3 c.1382C>T; p.P461L	EXTL3 c.1382C>T; p.P461L	EXTL3 c.1382C>T; p.P461L	EXTL3 c.1382C>T; p.P461L	EXTL3 c.1970A>G; p.N657S	EXTL3 c.1970A>G; p.N657S

n.d. means no data

Table S1. Overview clinical features of all nine affected individuals

Case	Infections	Infectious complication	Immunization	Allergy / Autoimmunity	HSCT (date)	Death
A:II-1	CMV disease	Respiratory failure	None	No	-	Yes (2mo)
B:II-1	Pneumonia 2x (X-ray proven) Enteritis (no pathogen)	-	Yes Incl. MMR: rubella IgG+ mumps IgG+ measles none	No allergy; Omenn like syndrome ^a	-	Alive
B:II-2	Pneumonia at 3 months	-	not before HSCT	None	Yes (2014)	Alive
C:II-1	-	-	Yes incl MMR	None	-	Alive
D:III-1	-	-	Yes incl MMR	None	-	Alive
D:III-2	-	-	Yes incl MMR	None	-	Alive
D:IV-1	-	-	Yes incl MMR	None	-	Alive
E:II-1	Omphalitis at 1 st week Septicemia at 2 months (both <i>S. aureus</i>)	Respiratory failure	No	No allergy; Omenn like syndrome	-	Yes (6mo)
E:II-2	- ^b	Respiratory failure	No	No allergy; Omenn like syndrome	-	Yes (10mo)

Table S2. Clinical features related to immunity in the *EXTL3*-mutated individuals.

^a In B:II-2, E:II-1, E:II-2, erythroderma at the age of 1, 4 and 6 months was diagnosed with a concomitant oligoclonal expansion (maternal cells have been excluded in all three). The erythroderma and clinical deterioration was most of immunological origin with a concomitant eosinophilia of 20-30% (>1500/ μ l).

^b Prophylactic antibiotics and IgG suppletion was shortly after birth initiated when the diagnosis of a SCID-related PID syndrome was confirmed in E:II-2.

Chr	HG19 position	Gene name	Gene ID	Variant	Amino acid change	PhyloP	Grantham score	ExAC frequency
chr8	g.28575113	<i>EXTL3</i>	NM_001440	c.1537C>T	p.Arg513Cys	6.238	180	NP
chr15	g.44949329	<i>SPG11</i>	NM_025137	c.833T>A	p.Asn278Ile	-0.137	149	2.00E-04
chr18	g.24496422	<i>CHST9</i>	NM_031422	c.1133A>G	p.Asn378Ser	5.264	46	3.40E-04
chr6	g.31106501	<i>PSORS1C1</i>	NM_014068	c.118insC	p.His40Profs*3	-1.310	1000	1.45E-01
chr6	g.31106501	<i>PSORS1C1</i>	NM_014068	c.118delC	p.His40Thrfs*25	-1.310	1000	1.16E-01
chr8	g.110425735	<i>PKHD1L1</i>	NM_177531	c.2321C>A	p.Thr774Asn	-0.135	65	NP
chr8	g.110492250	<i>PKHD1L1</i>	NM_177531	c.9209C>T	p.Pro3070Leu	4.438	98	NP
chr10	g.5781967	<i>FAM208B</i>	NM_017782	c.1834A>C	p.Thr612Pro	0.209	38	2.20E-03
chr10	g.5788833	<i>FAM208B</i>	NM_017782	c.3449C>G	p.Ser1150Cys	1.175	112	2.15E-03
chr11	g.62285208	<i>AHNAK</i>	NM_001620	c.16681T>G	p.Leu5561Val	-0.216	32	4.86E-04
chr11	g.62294937	<i>AHNAK</i>	NM_001620	c.6952G>A	p.Asp2318Asn	4.958	23	NP
chr16	g.68225292	<i>NFATC3</i>	NM_173165	c.2720C>T	p.Ser907Leu	4.016	145	9.06E-05
chr16	g.68260321	<i>NFATC3</i>	NM_173165	c.3175G>T	p.Ala1059Ser	-0.851	99	3.49E-04

Table S3. Homozygous and compound heterozygous variants detected by whole-exome sequencing in family A that segregate with the disease. NP means not present in database

Chr	HG19 position	Gene name	Gene ID	Variant	Amino acid change	PhyloP	SIFT score	ExAC frequency
chr5	g.73144828	<i>ARHGEF28</i>	NM_001080479	c.1663C>T	p.Arg555Cys	5.741	0.05	3.41E-04
chr8	g.28575584	<i>EXTL3</i>	NM_001440	c.2008T>G	p.Tyr670Asp	7.984	0	NP

Table S4. Homozygous variants shared by affected siblings in family B detected by whole-exome sequencing that segregate with the disease. NP means not present in database

Chr	HG19 position	Gene name	Gene ID	Variant	Amino acid change	PhyloP	SIFT	Polyphen-2 score	ExAC frequency
chr1	g.36638146	<i>MAP7D1</i>	NM_001286365	c.542G>A	p.Arg181Gln	3.743	0.01	0.927	7.10E-03
chr3	g.38149902	<i>DLEC1</i>	NM_007335	c.3025G>A	p.Gly1009Arg	5.330	0.06	0.999	6.05E-05
chr3	g.38149943	<i>DLEC1</i>	NM_007335	c.3066A>T	p.Lys1022Asn	-0.163	0.5	0.014	5.80E-03
chr8	g.28574958	<i>EXTL3</i>	NM_001440	c.1382C>T	p.Pro461Leu	7.728	0	1	1.65E-05
chr15	g.43017376	<i>CDAN1</i>	NM_138477	c.3524G>A	p.Cys1175Tyr	0.679	0.25	0.003	7.00E-04
chr15	g.43028677	<i>CDAN1</i>	NM_138477	c.392G>A	p.Arg131His	2.534	0.03	0.871	n.p.
chr18	g.33795696	<i>MOCOS</i>	NM_017947	c.1553A>G	p.Asp518Gly	-0.021	0.33	0.013	n.p.
chr18	g.33840056	<i>MOCOS</i>	NM_017947	c.2327G>A	p.Arg776His	6.685	0	1	5.77E-05
chr18	g.65181049	<i>DSEL</i>	NM_032160	c.827A>G	p.Asn276Ser	4.055	0.4	0.004	1.37E-02

Table S5. Homozygous and compound heterozygous variants detected by whole-exome sequencing in family C that segregate with the disease. n.p. means not present in database

Chr	HG19 position	Gene name	Gene ID	Variant	Amino acid change	PhyloP	SIFT score	Polyphen-2 score	ExAC frequency
chr8	g.27151632	<i>TRIM35</i>	NM_171982	c.727A>T	p.Met243Leu	-0.050	0.29	0	5.10E-03
chr8	g.27462662	<i>CLU</i>	NM_001831	c.698C>T	p.Thr203Ile	1.742	0.37	0.017	1.67E-03
chr8	g.28574958	<i>EXTL3</i>	NM_001440	c.1382C>T	p.Pro461Leu	7.727	0	1	1.65E-05

Table S6. Homozygous variants shared by affected siblings in family D detected by whole-exome sequencing that segregate with the disease.

Chr	HG19 position	Gene name	Gene ID	Variant	Amino acid change	PhyloP	SIFT score	Polyphen-2 score	ExAC frequency
chr1	g.228336425	<i>GUK1</i>	NM_1242840	c.598G>A	p.Gly200Arg	0.222	0.02	0	1.70E-03
chr8	g.28575546	<i>EXTL3</i>	NM_001440	c.1970A>G	p.Asn657Ser	9.206	0	0	5.80E-05
chr8	g.68092107	<i>CSPP1</i>	NM_024790	c.3151G>A	p.Asp1051Asn	1.191	0.16	0.474	4.15E-05
chr1	g.201618182	<i>NAV1</i>	NM_020443	c.386G>A	p.Gly129Asp	1.249	0.01	0.215	3.43E-05
chr1	g.201779219	<i>NAV1</i>	NM_020443	c.4547C>T	p.Ser1516Leu	1.115	0.28	0	6.60E-05
chr6	g.90382388	<i>MDN1</i>	NM_014611	c.13508A>G	p.Asp4503Gly	3.367	n.a.	0.276	1.57E-04
chr6	g.90453354	<i>MDN1</i>	NM_014611	c.4258A>G	p.Met1420Val	6.159	n.a.	0.688	2.24E-03

Table S7. Homozygous and compound heterozygous variants shared by affected siblings in family E detected by whole-exome sequencing that segregate with the disease.