Supplementary Content

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Supplementary Results

Clinical history of patients harboring *XIAP* variants

Patient 1 (XIAP E99X)

A Caucasian male patient presented with bloody diarrhea and anal fissure at the age of nine months. Initially, cow's milk protein intolerance was suspected. However dietary exclusion of milk protein did not resolve symptoms. At the age of 19 months, a colonoscopy was performed, which demonstrated severe, erosive, discontinuous pancolitis with anal fissures and anal stenosis and histological demonstration of superficial, erosive, and ulcerative inflammation with crypt abscesses but without epithelioid granulomas. Histologic and serologic testing for EBV, CMV, and mycobacteria was negative. The family history was negative for immunodeficiency syndromes and inflammatory bowel disease. The subsequent course of disease was refractory to treatment with local and oral mesalazine, corticosteroids, antibiotics, probiotics, and with intolerance to azathioprine (pancreatitis). Anal dilatation was associated with symptomatic relief. Colonoscopy at 31 months of age revealed a similar picture of moderate to severe colitis, anal fissures, and anal stenosis. Upper endoscopy and MR enterography did not reveal signs of macroscopic small intestinal or upper gastrointestinal disease. However, histologic examination demonstrated low-grade, chronic, *Helicobacter pylori*-negative gastritis and duodenitis in accordance with upper gastrointestinal CD. An MRI revealed hepatosplenomegaly. In addition, papular and pustular skin manifestations were found at the back and thighs with histiocytic infiltrates and epithelioid cells in accordance with a cutaneous CD manifestation. Following primary failure of infliximab, the patient received a loop ileostomy at the age of 36 months, which was associated with significant clinical improvement despite persistent mild, discontinuous colitis. At 40 months of age, surgical treatment of an anocutaneous fistula was performed. Since then, the patient has been in clinical remission for more than 17 months without treatment. Self-limiting episodes of fever have occurred, but never fulfilled the criteria of hemophagocytic lymphohistiocytosis (HLH). The patient continues to be negative for EBV. A summary of laboratory findings is shown in Table S2 and S3. The patient was

classified as A1a, L2 + L4a, B2p according to the Paris modification of the Montreal classification [1].

Patient 2 (XIAP G39C)

Patient 2 is a male Caucasian with CD and an indicated age of onset at 13 years, who was enrolled in an infliximab study at the age of 38 years. Further clinical information on this patient was not available.

Patient 3 (XIAP K297T)

In a Caucasian male patient, the diagnosis of CD was made at the age of 16 years after a one year history of abdominal pain, diarrhea, and weight loss of 10 kg (onset of disease at the age of 15 years). Endoscopy of the upper and lower gastrointestinal tract showed discontinuous, ulcerative inflammation of the jejunum, terminal ileum, left colon, sigmoid colon, and rectum as well as of the gastric antrum with mild pyloric stenosis (Fig. S5). Perianal disease was mild with skin tags but no fistulae. Histology showed multiple granulomas in all inflamed parts of the gastrointestinal tract including the gastric antrum. Disease classification according to the Paris modification of the Montreal classification for CD [1] was A1b, L3+L4a/L4b, B2p. The patient was tested positive for ASCA IgA and negative for ANCA antibodies. The personal and family history were negative for immunodeficiencies and inflammatory bowel disease.

Under an exclusive enteral nutrition for 6 weeks, clinical remission was achieved, although laboratory values (elevated C-reactive protein and fecal calprotectin) still showed disease activity. The patient intermittently required corticosteroid treatment. Azathioprine treatment was initiated at the age of 16 years. At the age of 17 (18 month after the diagnosis of CD was made), infliximab was initiated in addition to azathioprine. At the age of 19, infliximab intervals needed to be reduced to 4 weeks. The patient continued to require intermittent corticosteroid treatment (budesonide) and

infusions of iron (ferric carboxymaltose). Currently, the patient is in stable condition and receives infliximab (4-weekly interval), azathioprine, mesalamine, pantoprazole, zinc, vitamin d, folic acid and supplemental enteral nutrition.

The patient was tested negative for three CD-associated variants of NOD2: R702W (SNP8), G908R (SNP12), 3020insC (SNP13).

Patient 4 (W323X)

Patient 4, a male Caucasian, was diagnosed with CD at the age of 16 years in 1966 (A1b, L3, B2B3p according to the Paris modification of the Montreal classification [1]). The family history was negative for IBD. Due to fulminant colitis, the patient underwent colectomy with ileostomy at the age of 16 years. A stenosing and fistulizing course of ileitis required several consecutive ileal resections in the first year following diagnosis and again at the age of 29 years (1979). From the time of diagnosis until the age of 52, the patient received corticosteroids on a continuous basis. Azathioprine was initiated at the age of 50 but was discontinued after 8 months of treatment due to severe leukopenia associated with recurrent and persistent respiratory infections. The patient reported at our center at the age of 53 (2003), when infliximab treatment was initiated due to severe ileitis with enterocutaneous fistulas. Infliximab was discontinued after 4 months of treatment due to severe pneumonia requiring mechanical ventilation but with negative microbiological testing including negative results for *M. tuberculosis*. After recovery, the patient underwent resection of a stenotic jejunal segment with an enterocutaneous fistula. Following surgery, the patient acquired a nosocomial pneumonia and died at the age of 54. The patient was tested negative for EBV at several occasions.

Supplementary Methods

Cell lines

XIAP-deficient and -sufficient HCT116 cells were a gift from B. Vogelstein (Johns Hopkins University) [2]. HCT116 and 293T cells were cultured in RPMI-1640 containing 10% FCS.

Flow cytometry

Flow cytometry was performed as described previously [3]. Briefly, cells were staining with monoclonal antibodies for 20 min at 4°C. For detection of iNKT cells, staining with PBS57-loaded CD1d tetramers (1h, 4°C, NIH Tetramer Core Facility) was performed. Data were collected using a BD Biosciences FACSVerse and were analyzed by Flowjo (Tree Star, Inc., Ashland, OR). For intracellular staining, cells were first surface-stained, then permeabilized with Cytofix/Cytoperm (BD Biosciences) and washed with Perm/Wash buffer (BD Biosciences) according to the manufacturer's instructions. Antibodies were added for 30 min at 4°C before washing with Perm/Wash buffer. Antibodies and reagents used for flow cytometry were obtained from BD Biosciences except for CD1d-PBS57 tetramers (NIH Tetramer Core Facility). Via-Probe (BD Biosciences), and in case of intracellular staining, fixable viability dye eFluor 450 (eBioscience, Frankfurt, Germany), was added to all stainings for exclusion of dead cells. XIAP clone 48 (BD Biosciences) was used for detection of intracellular XIAP according to [4].

Western blotting and immunoprecipitation

Immunoprecipitation was performed as described previously with minor modifications [5]. Briefly, 293T cells were transfected using Lipofectamine 2000 (Invitrogen) with Flag-RIPK2 together with either empty vector (pcDNA), mycXIAPwt or mutant mycXIAP. 24h after transfection, the cells were lyzed and protein complexes were immunoprecipitated using anti-myc antibody, followed by SDS-PAGE and immunoblotting using anti-myc and anti-Flag antibodies. Western blotting was performed as described previously [5]. The following antibodies were used: anti-myc (Roche),

anti-flag (M2, Sigma-Aldrich), rabbit anti-caspase-1 (D7F10; Cell Signaling Technology), mouse anti-XIAP (clone 48, BD Biosciences), mouse anti-β-actin (Sigma-Aldrich).

Monocyte-derived DCs and lentiviral reconstitution

Monocytes were extracted from PBMCs by positive selection using CD14 magnetic beads (Miltenyi Biotec) and were cultured at $1x10^6$ cells/ml for 5 days in complete RPMI-1640 medium supplemented with 500 U/ml recombinant hIL-4 and 1000 U/ml recombinant hGM-CSF (PeproTech). Lentiviral production and transduction was performed as described previously [3]. Briefly, lentiviral particles were generated in 293T cells using pCMVΔR8.9 and pMD2.G [6]. Lentiviral particles were concentrated by ultracentrifugation and titered by GFP expression in 293T cells. On day 3 after isolation of monocytes, monocyte-derived DCs were infected with lentiviruses expressing XIAP and GFP [5] or a control virus expressing GFP only at an MOI of 20 in RPMI-1640 containing 10 μg/ml protamine sulphate (Sigma Aldrich). Immature transduced DCs were cultured at $1x10^6$ cells/ml until day 5 when they were investigated in functional studies.

Supplementary Figures 1-6

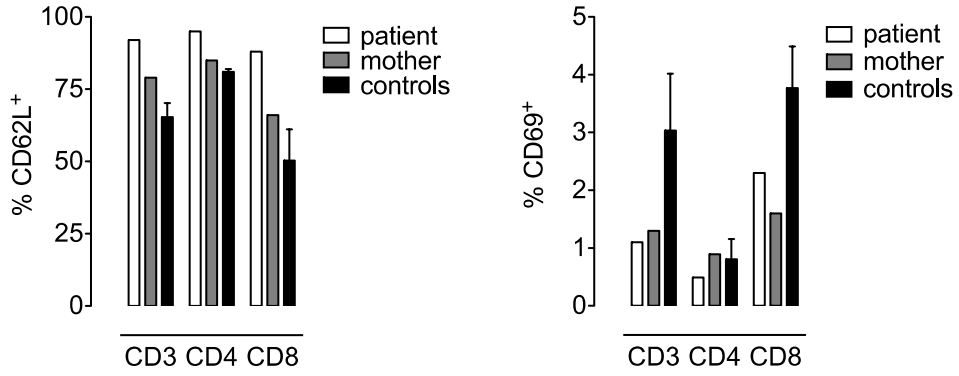

Figure S1. Naive T cell profile in the XIAP E99X patient (Patient 1)

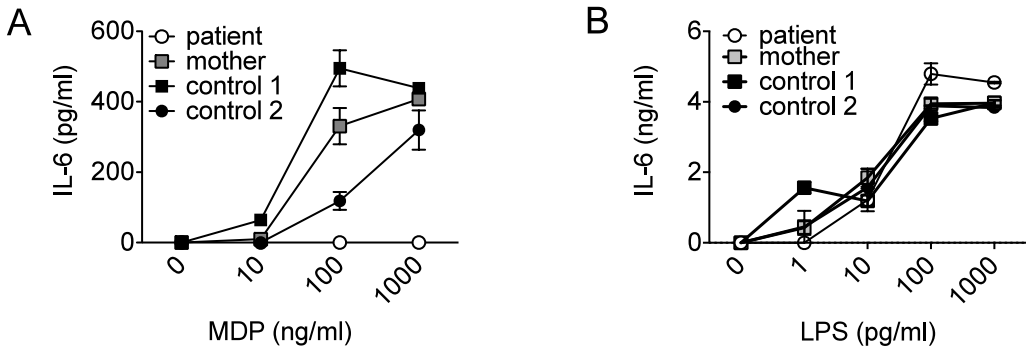

Figure S2. A severe and selective defect in NOD2-dependent IL-6 secretion in the XIAP E99X patient (Patient 1)

IL-6 secretion as determined by ELISA in supernatants of PBMCs treated with the MDP (A) or LPS (B) for 24h. Mean ± s.e.m. of triplicate cultures are shown.

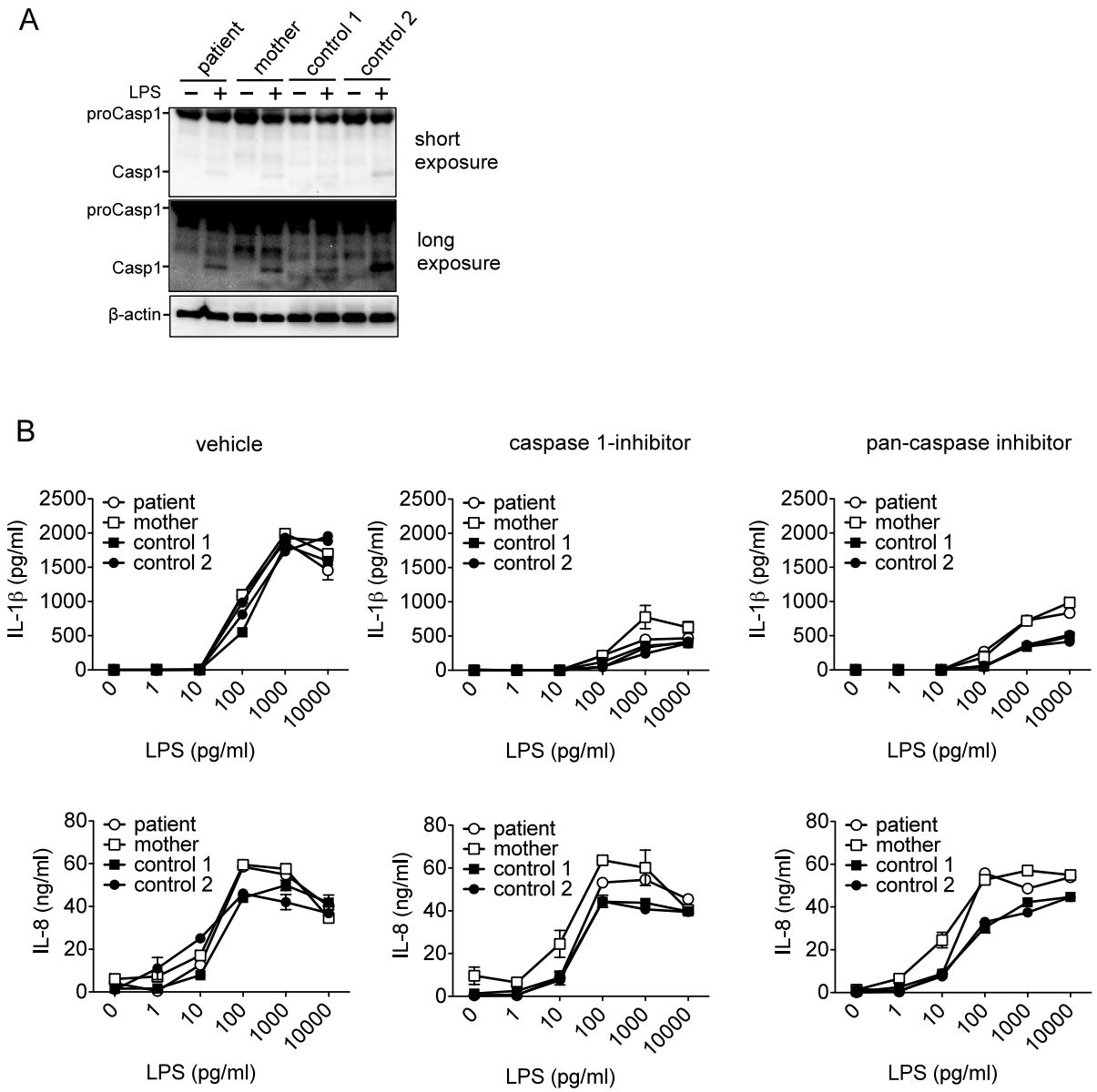

Figure S3. Unaltered caspase-1 cleavage and IL-1β secretion in the XIAP E99X patient (Patient 1)

A: Caspase-1 cleavage in primary PBMCs induced by treatment with 10 ng/ml LPS for 8 hours as detected by western blotting. B: IL-1β (upper panel) and IL-8 (lower panel) secretion by PBMCs upon stimulation with LPS at the indicated concentration as determined by ELISA. Where indicated, caspase-1 inhibitor (middle panels, Z-YVAD-fmk, 10 μM) or pan-caspase inhibitor (right panels; Z-VAD-fmk, 10 μM) was added to confirm that Il-1β secretion is mediated by caspase-1. In B, mean ± s.e.m. of triplicate cultures is shown.

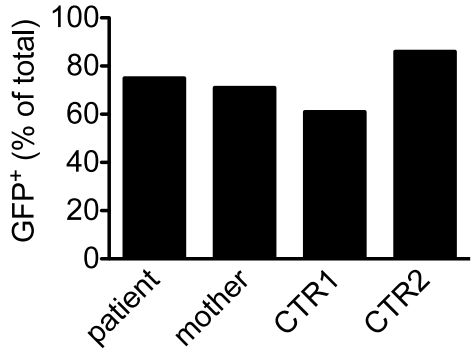

Figure S4. Lentiviral transduction rates of monocyte-derived dendritic cells (moDCs)

The percentage of GFP-positive moDCs 48h after transduction with lentiviruses expressing XIAP is indicated.

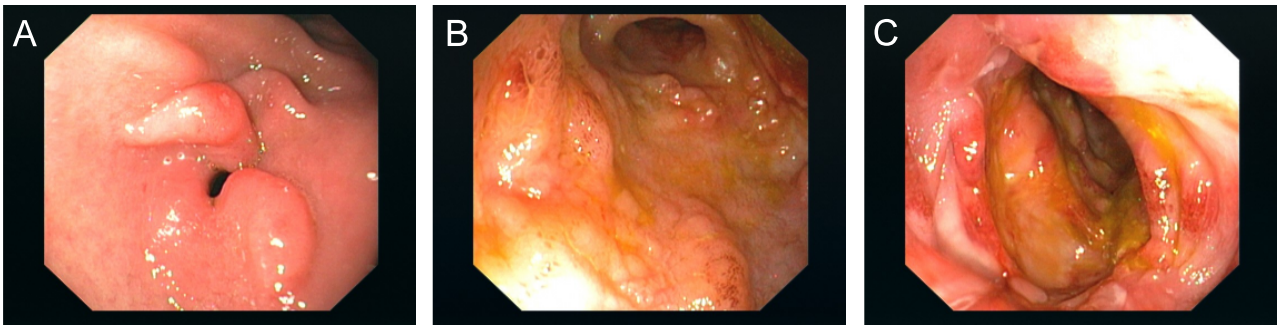

Figure S5. Images of upper and lower gastrointestinal endoscopy of patient 3 (XIAP K297T).

A. Gastric antrum with pyloric stenosis. B. Terminal ileitis. C. Colitis of the sigmoid colon.

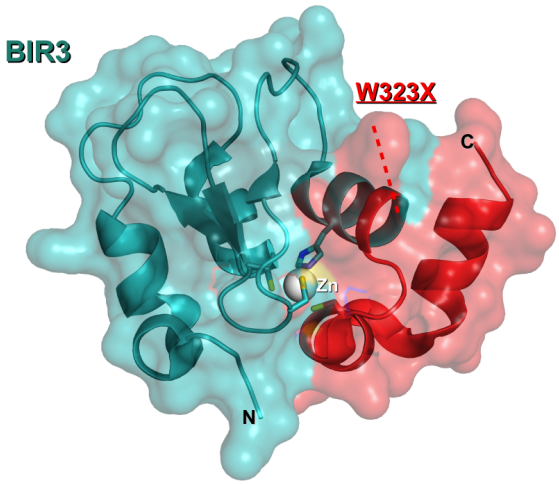

Figure S6. Structural model illustrating the W323X variant (Patient 4). Cartoon and surface representation of the XIAP BIR3 protein domain (PDB ID 2pop, chain B, residues 265-330). Truncation by W323X is indicated by a dashed line and deleted substructures are colored in red. W323X removes the C-terminus of BIR3 including one zinc-coordinating cysteine crucial for domain stability. The mutation likely impairs BIR3 function by preventing proper folding of the domain.

Supplementary Tables 1-5

Table S1. Primer and PCR conditions for Sanger sequencing of *XIAP*.

Primers

Target	Primer name	F-primer	R-primer
Exon 2, part 1	E2a	TTGCTTGTGTTTCACATTTTG	GTCTGGCCAGTTCTGAAAGG
Exon 2, part2	E2b	TTGAAAATAGTGCCACGCAG	CCATTTTGCTATTGGGGATG
Exon 3	E3	TCTGGGGAACAGAGCCAG	TTTCAAGCCATAGTGAAGGC
Exon 4	E4	TGGGATAGGGAATTGGGTAAC	ACACTGCCCAGCTAGCTCTC
Exon 5	E5	TCCTTCTAGCTCCATCAGGC	CGCAGGCCTGTAGTCCC
Exon 6	E6	GCTTTGCTTTGCTGGTTTG	TGTCCATATCCACCTGTCCC
Exon 7	E7	ACTTGTTGGGTGCTTGGC	CTTGGTAGCAAATGCTAATGG

PCR temperature profile

95°C	12 min	
95°C 60-65°C 72°C	30 sec 30 sec 30 sec	35 cycles
72°C 10°C	10 min ∞	

Annealing temperatures

60°C	E2a, E2b
63°C	E3, E6, E7
65°C	E4, E5

Table S2. Summary of microbiological testing of patient 1 (XIAP E99X).

Patient age (months)	**20**	**20**	**24**	**24**	**33**	**40**
Material	blood	stool	rectum biopsy	stool	blood	stool
CMV PCR			neg		neg	
Adenovirus PCR				neg		neg
Norovirus PCR		neg		neg		neg
Rotavirus PCR				neg		neg
Enteropathogenic bacteria culture		neg		neg		neg
EBV-IgG	neg				neg	
EBV-IgM	neg				neg	
EBV-PCR	neg					
Atypical mycobacteria - PCR			neg			

neg = negative; gray fields indicate data not available

Table S3. Summary of clinical laboratory data from patient 1 (XIAP E99X).

Pat. age (months)	19	20	22	27	31	34	38	40	53	Reference
Hb (g/dl)	9.1	10.2		13.1		11.1	15.4		10.2	10.7-13.9
Hkt (%)	27.6	31.5		38.4			46.5		32.9	32.5-41.5
MCV (fl)	76	74		74			67		65	74-89
Leukocytes (/nl)	16.3	7.1		12.7		12.0	10.7	7.5	11.7	5.1-12.9
Lymphocytes (/µl)	11224	7106								3000-9500
$CD3^+$ cells (/µl)				3103						1800-3000
$CD4^+$ cells (/µl)				2288						700-1800
$CD8^+$ cells (/µl)				720						500-1500
CD4/CD8 ratio				3.18						0.865-1.885
Natural Killer cells (/µl)				247						200-600
$CD19^+$ cells (/µl)				804						300-1300
Monocytes (/µl)				797						50-1000
Eosinophils (/µl)		258								40-650
Basophils (/µl)		129		159						0-70
Thrombocytes (/nl)	287	135		330		428			277	286-509
CRP (mg/l)		3.9				11.5		2.1	19.2	< 5
Antibodies & functional analysis										
IgA (g/l)	0.533			0.857						0.3-1.2
IgG (g/l)	6.61			10.2						3.5-10
IgM (g/l)				1.86						0.4-1.4
IgG1 (g/l)	4.36			7.3						2.4-7.8
IgG2 (g/l)	0.417			0.627						0.55-2
IgG3 (g/l)	0.087			0.225						0.15-0.93
IgG4 (g/l)	0.015			0.046						0.006-0.69
IgE (kU/l)	41.3									< 100
anti-Transglutaminase IgA (kU/l)	2.1									0-9
anti-Transglutaminase IgG (kU/l)	1.8									0-9
ANA			neg							
pANCA			neg							
cANCA			neg							
anti-myeloperoxidase			neg							
anti-proteinase			neg							
anti-lactoferrin			neg							
anti-elastase			neg							
anti-cathepsin G			neg							
anti-lysozyme			neg							
anti-mitochondrial			neg							
ASCA IgG			pos							
ASCA IgM			pos							
SAP staining					n					
Oxidative burst					n					
CD107 degranulation					n					
Stool										
Calprotectin (mg/kg)						592				
Elastase (µg/g stool)								280		> 200

n = normal, neg = negative, pos = positive, pat. = patient; gray fields indicate data not available

Table S4. Exome sequencing coverage (patient 1)

Sample	Coverage of exome target				
	% covered ≥1x	% covered ≥8x	% covered ≥20x	mean coverage	Gb of coverage
father	96.98	92.87	86.88	69.45	4.31
mother	97.17	93.67	88.78	80.54	5.00
child	96.38	91.02	80.72	44.10	2.74

Table S5. Information on variants in *FAM151A*, *RAPGEF4*, and *DCHS2* (patient 1, XIAP E99X)

Gene	Type	Exonic Function	Status in son	Status in parents	Amino Acid Change	SIFT	Poly-Phen2	PhyloP	Mutation Taster
FAM151A	exonic	frameshift deletion	homozygous	both parents het	NM_176782:c.1003_1031del:p.335_344del				
RAPGEF4	exonic	nonsynonymous SNV	heterozygous	only father het	NM_007023:c.G215A:p.R72H	0,14	1	1,000	1
RAPGEF4	exonic	nonsynonymous SNV	heterozygous	only mother het	NM_001100397:c.G1504T:p.V502F	0,27	0,05	1,000	0,9953
DCHS2	exonic	nonsynonymous SNV	heterozygous	only father het	NM_017639:c.G7513A:p.A2505T	0	0,980	0,999	0,4202
DCHS2	exonic	stopgain SNV	heterozygous	only mother het	NM_017639:c.T6155G:p.L2052X	0,75	0,565	0,844	1
DCHS2	exonic	nonsynonymous SNV	heterozygous	only mother het	NM_017639:c.G4561T:p.A1521S	0,92	0	0,149	2,70E-05

Gene	Chr	Start	End	Obs	Ref
FAM151A	chr1	55076138	55076166	-	CACTCCACATTCAGACCGTCATCCCCAGG
RAPGEF4	chr2	173662259	173662259	A	G
RAPGEF4	chr2	173882160	173882160	T	G
DCHS2	chr4	155156926	155156926	T	C
DCHS2	chr4	155158284	155158284	C	A
DCHS2	chr4	155219540	155219540	A	C

Supplementary References

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