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Supplementary Information for

Homozygous *NLRP1* gain-of-function mutation in siblings with a syndromic form of recurrent respiratory papillomatosis

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SUPLEMENTARY CASE REPORTS

P1, 9 years old at time of manuscript preparation, was born at term after an uneventful pregnancy. Until the age of 5, his weight and length growth were below 25th percentile; however after age of 5, growth has been closer to 75th percentile. He has mild delayed psychomotor development. Hoarseness and recurrent laryngitis developed at age 2 years, and direct laryngoscopy revealed papillomas on the glottis and supraglottis. He required 10 surgical procedures over the next year. From age 5-9, he had had 30 laser ablations for recurrent laryngeal papillomatosis. P1 developed mild dermatologic abnormalities shortly after birth including keratosis pilaris on the legs and arms since birth, atrophoderma vermiculata on the cheeks, and warts on hands and feet. Additionally he has Legg-Calvé Perthes of the right hip, and cow milk allergy. At the age of 4 years old, he presented acute osteomyelitis of the left talus that required intravenous antibiotics. HIV testing was negative.

Immunologic workup:

- Normal WBC differentiation, normal B and T cell subsets; increased memory CD4 and CD8 T cells
    - CD3+/CD4+: CD45RO+ (memory): 48% CD45RA+ (naïeve): 46%
    - CD3+/CD8+: CD45RO+ (memory): 44% , CD45RA+ (naïeve): 58%
- Normal lymphoproliferation against PHA, mild against Candida and tetanus.
- Normal total IgG, IgG subclasses, normal IgM, IgA, IgE (200- 300 kU/lL).
- NK cell cytotoxicity: mildly decreased CD107a and IFNg expression after stimulation, normal NK cell killing

P2, 5 years old at time of manuscript preparation, was born at term after an uneventful pregnancy. He had ongoing weight and length growth stagnation (below 3rd percentile until the age of 2 years), and currently weight and length are at the 3rd percentile. Psychomotor development was also delayed. P2 developed hoarseness shortly after birth. Laryngoscopy revealed papillomas on the larynx, less severe than P1. He required 2-3 laser ablations of these papillomas each year until last year. Shortly after birth he developed skin abnormalities similar to P1 including Keratosis Pilaris on the legs and arms and atrophoderma vermiculata on the cheeks, and multiple hyperpigmented lesions on the buttocks. Additionally he has been noted to have a high palette, and has bilateral knee flexion contractures without evidence of any joint inflammation. HIV testing was negative.

Immunologic workup:

- Normal WBC differentiation, normal B and T cell subset and maturation
- Lymphoproliferation: normal against PHA, mild against Candida, absent against tetanus.
- Normal total IgG, IgG subclasses, normal IgM en IgA, IgE (229 kU/lL),
- Normal Polysaccharide antibody response (after Pneumo23® vaccine), normal immunity against HSV, Tetanus
- NK cell cytotoxicity: mildly decreased CD107a and IFNg expression after stimulation, normal NK cell killing

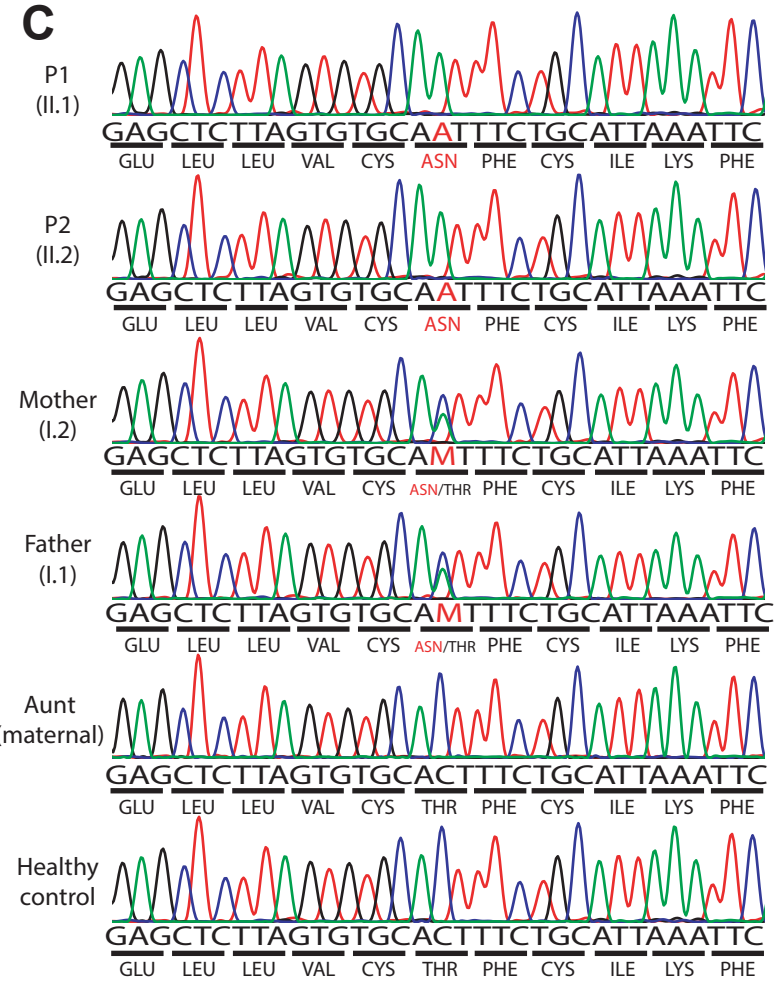
A

	Number of annotated variants	
	P1	P2
Total	163,409	164,543
Homozygous	68,962	69,959
Non-synonymous, essential spicing, or indel	4,721	4,624
MAF < 0.01 (1%) in ExAC, 1000KG, ESP	79	70
Shared by P1 and P2 and heterozygous in parents	11	
Remove pipeline artifacts (inhouse MAF >0.01)	5	

B

Chr	pos	Ref Nt	Alt Nt	Gene	AA change	ExAC MAF (het/hom)	Sift	Polyphen (2HVAR)	CADD (Phred_V1.3)	Gene function or known clinical associations
7	150644044	G	A	KCNH2	p.Pro1084Leu	0.00001711 (2/0)	D	P	23.1	Long QT syndrome 2, Short QT syndrome 1 (OMIM: 613688, 609620)
16	57931428	C	T	CNGB1	p.Gly1039Arg	0.003250 (390/1)	D	D	34	Retinitis pigmentosa 45 (OMIM: 613767)
17	5461752	G	T	NLRP1	p.Thr755Asn	private	D	D	23.1	Autoinflamation with arthritis and dyskeratosis, Multiple self-healing palmoplantar carcinoma (OMIM: 617388, 615225)
19	57867517	T	C	ZNF304	p.Cys94Arg	0.005495 (667/6)	D	B	13.83	C2H2-type zinc-finger, transcriptional regulator of B1 integrin
19	58420013	C	T	ZNF417	p.Glu545Lys	private	D	B	24.5	unknown

C



D

	Number of annotated variants	
	P1	P2
Total	163,409	164,543
X chromosome (hemizygous)	1,905	1,937
Non-synonymous, essential spicing, or indel	156	150
MAF < 0.0001 (0.01%) in ExAC, 1000KG, ESP	1	8
Shared by P1 and P2 and heterozygous in mother	0	
Remove pipeline artifacts (inhouse MAF >0.01)	0	

Figure S1. Filtering of annotated variants after whole exome sequencing. **A**. Number of variants after each filtering step used for a homozygous recessive genetic model. **B**. Selected details of the 5 variants after filtering as in A. **C.** Sanger sequencing of genomic DNA from selected individuals and control. **D**. Filtering of variants under an X-linked genetic model.

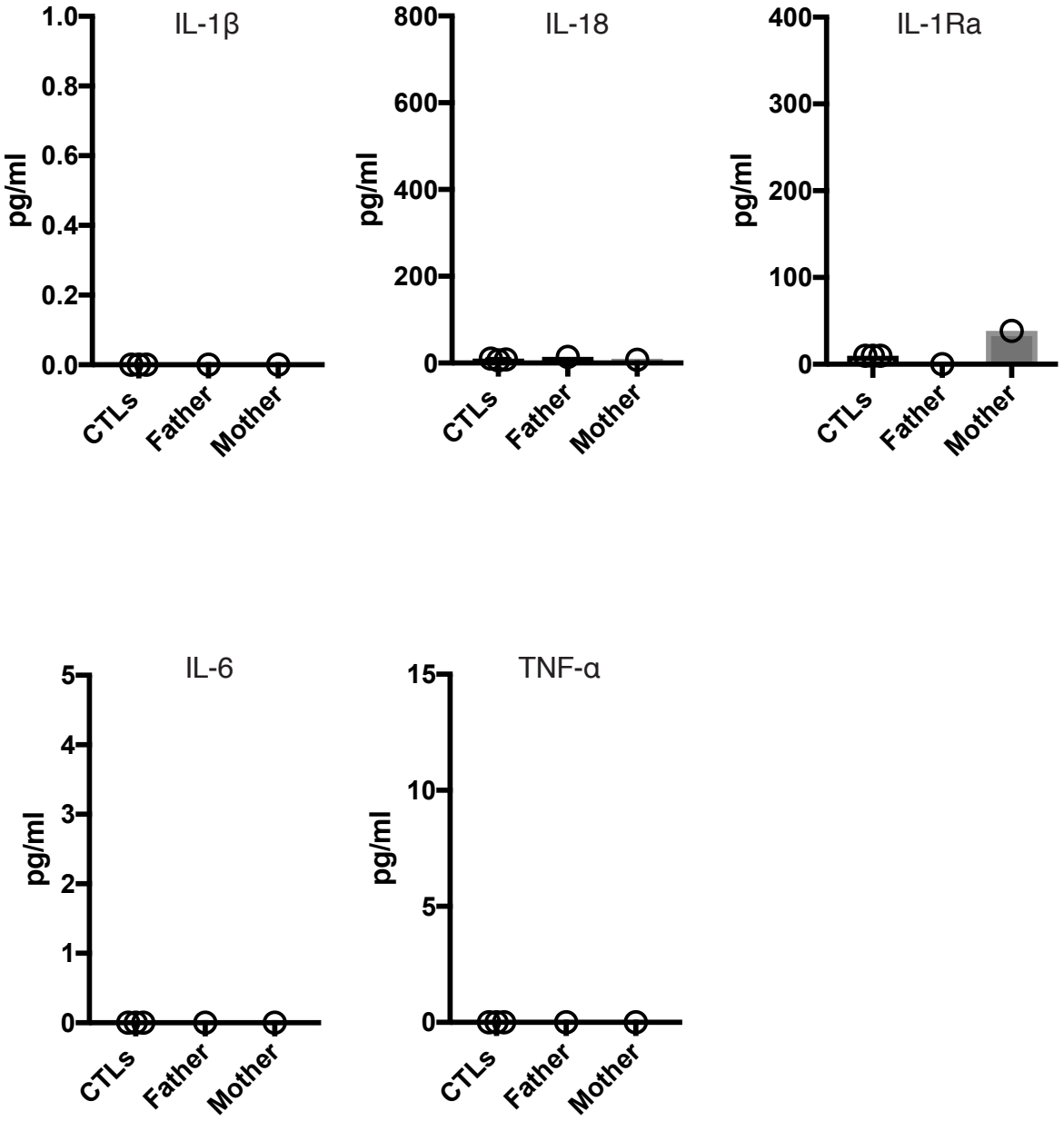


Figure S2. Serum cytokine measurements in heterozygous parents and healthy controls. LUMINEX measurements of serum levels of IL-1β, IL-18, IL-1Ra, IL-6 and TNF-α from Father (I.1), Mother (I.2) and 3 healthy controls. Results are representative of 2 independent experiments.

Cloning

NLRP1.F	atggctggcggagcctggggccg
NLRP1.R	tcagctgctgagtggcaggagtccctttttgctgccc

Subcloning

NLRP1.3'.XhoI.pcDNA3.1	GTTTAAACGGGCCCTCTAGActcgagtcagctgctgagtggcagg
NLRP1.5'.EcoRI.pcDNA3.1	TCCACTAGTCCAGTGTGGTGGAATTCGCCACCatggctggcggagcctgg
NLRP1.T755N join F	tcttagtgtgcaAtttctgcattaa
NLRP1.T755N join R	ttaatgcagaaaTtgcacactaaga
NLRP1.WT755 join F	tcttagtgtgcactttctgcattaa
NLRP1.WT755. join R	ttaatgcagaaagtgcacactaaga

Sanger sequencing

NLRP1.370.F	ccaccgcagtgctaatgc
NLRP1.370.R	caaaacccttctctctgagc
NLRP1.740.F	cacaggcgcacacc
NLRP1.740.R	gggccatgtggaacagag
NLRP1.1110.F	gaccgcttccagcatg
NLRP1.1110.R	ctcagcgagactcaccac
NLRP1.1480.F	ctctgagtccagcaggaag
NLRP1.1480.R	gaccaacctaaaggctctaattg
NLRP1.1850.F	ccttcttgaagatgggtattcttc
NLRP1.1850.R	gcaaagaactcttggaaacag
NLRP1.2220.F	ggaacaaaacgttcctgacac
NLRP1.2220.R	cactaagagctccatgtctg
NLRP1.2590.F	gctgaggactgcaaggac
NLRP1.2590.R	gcacattgaagctcaggtc
NLRP1.2960.F	ggagcaggagaaacctcag
NLRP1.2960.R	gtatccaggccctcagtag
NLRP1.3330.F	gctactgaggtagttgacaaag
NLRP1.3330.R	gttgggccagcggtag
NLRP1.3700.F	gatccataatgccctgcg
NLRP1.3700.R	gtgacttcctcaggatggac
NLRP1.4070.F	gtgggaggccttggtg
NLRP1.4070.R	gctatgcgggctggag

Table S1: List of PCR primers used in this study