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Supplemental Data

Whole-Exome-Sequencing-Based Discovery of Human FADD Deficiency

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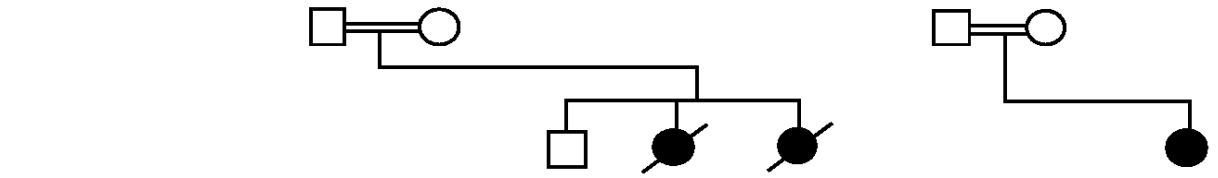

FADD mutation		C105 W/WT	C105W /WT	WT/WT	C105W /C105 W	C105W/ C105W	C105W /WT	C105W /WT	C105W /C105 W	
Individual		III : 1	III : 2	IV : 1	P2	P3	III : 4	III : 3	P4	
Marker	Physical position									
D11S4459	56514519	248-254	248-254	254-254	**248-248**	**248-248**	248-254	**248-248**	248-254	
D11S2006	59721888	317-321	317-321	317-317	**321-321**	**321-321**	317-321	317-321	317-321	
D11S4191	59999717					**100-100**		92-100	92-100	
D11S4076	61363135	148-154	**148-148**	148-154	**148-148**	**148-148**	**148-148**	**148-148**	**148-148**	
D11S1889	67313143	196-200	196-200	196-196	**200-200**	**200-200**	196-200	196-200	196-200	
D11S987	67893340	127-129	119-129	120-127	**129-129**	**129-129**	120-129	120-129	120-129	
D11S4087	68099158	**157-157**	**157-157**	**157-157**	**157-157**	**157-157**	**157-157**	**157-157**	**157-157**	
D11S4113	68765634	230-238	230-238	230-238	**230-230**	**230-230**	**230-230**	**230-230**	**230-230**	
D11S4136	69615711	187-189	178-189	178-187	**189-189**	**189-189**	178-189	178-189	**189-189**	
D11S4196	70185224	200-228	200-228	228-228	**200-200**	**200-200**	200-228	200-228	**200-200**	*FADD*
D11S4139	70504269	147-182	147-182	182-182	**147-147**	**147-147**	147-182	147-182	**147-147**	
D11S1314	72323249	**96-96**	**96-96**	**96-96**	**96-96**	**96-96**	**96-96**	**96-96**	**96-96**	
D11S2371	73505131		195-203	195-195	**203-203**	**203-203**	195-203	195-203	**203-203**	
D11S4081	75196447		185-187	187-187	**185-185**	**185-185**	185-187	179-185	**185-185**	
D11S4179	76396260	**235-235**	**235-235**	**235-235**	**235-235**	**235-235**	**235-235**	**235-235**	**235-235**	
D11S937	77854320	165-169	157-179	169-179	157-165	157-165	**157-157**	165-169	157-165	
D11S2002	79965403	235-243	231-235	235-235	231-243	231-243	**231-231**	235-243	231-243	
D11S901	81844785	**316-316**	320-324	216-320	316-324	316-324	**324-324**	316-324?	316-324	
D18S61	65587036	**215-215**	**215-215**	**215-215**	**215-215**	**215-215**	215-237	213-215	**215-215**	
ATA82B02	67064497	188-191	188-191	188-191	**191-191**	**191-191**	188-191	179-188	**188-188**	

Figure S1. Haplotypes of Microsatellites Sequenced Inside the Two Linked Regions on Chromosome 11 and Chromosome 18

18 microsatellites were sequenced in the chromosome 11 region and 2 in the chromosome 18 region. In bold, microsatellites with a homozygous haplotype. And inside frames are homozygous stretches found by linkage analysis. The black line is here to locate the position of FADD.

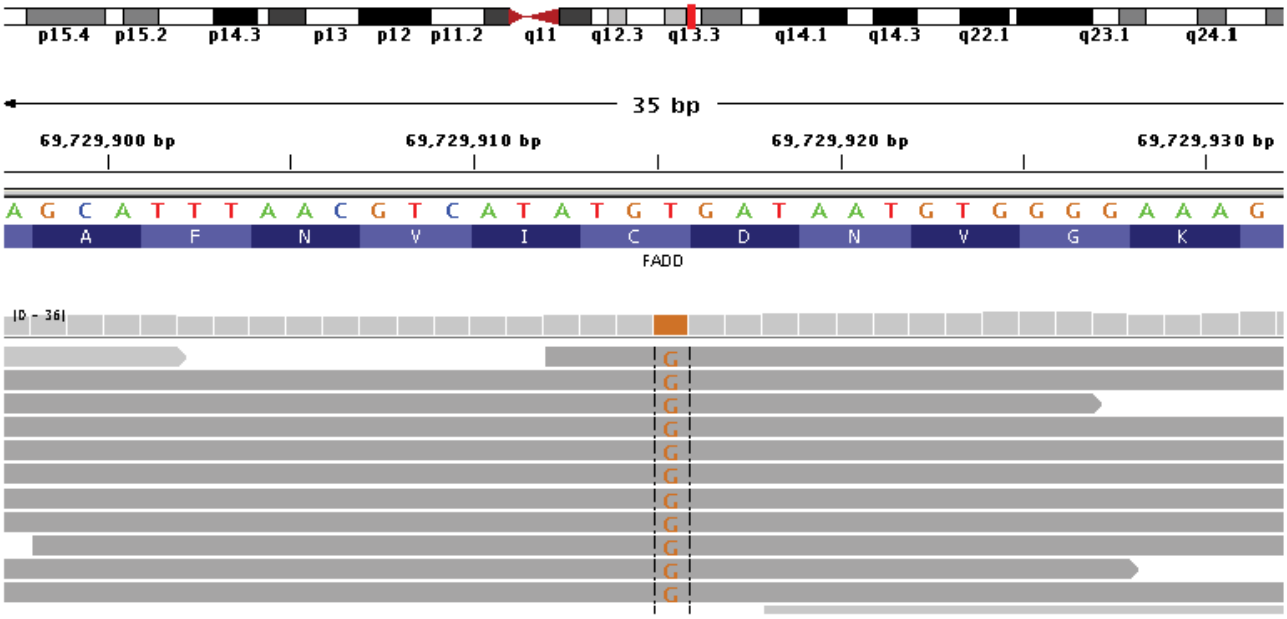

Figure S2. Illumina Sequencing Reads Displayed for Patient P3

Reads overlapping the mutation in exon 2 of FADD (bp position g.69,729,880 – g.69,729,949, hg18, NCBI 36.1) show the homozygous T>G base pair substitution.

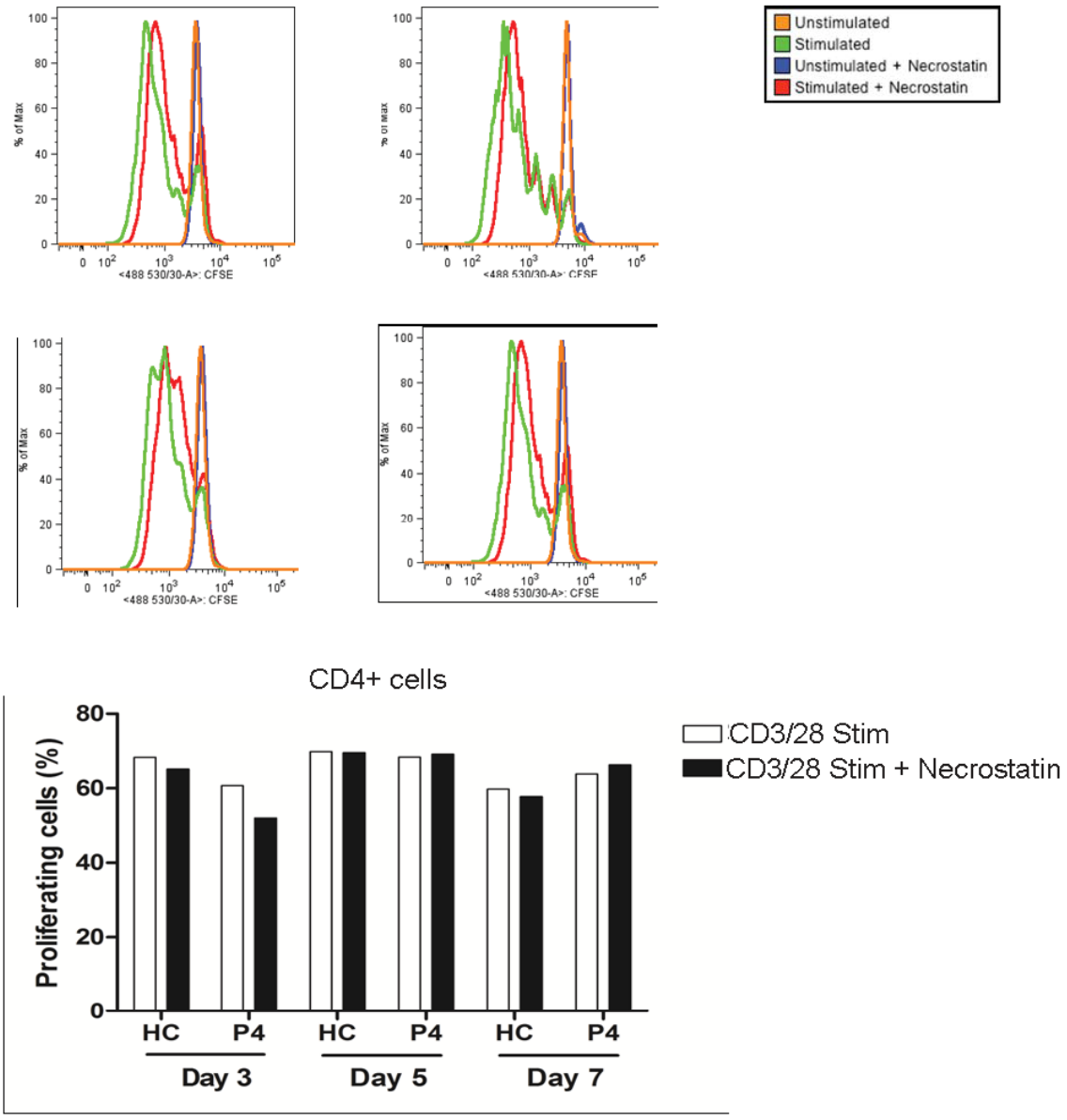

Figure S3. CFSE Proliferation Assay after CD3/28 Stimulation

Here results on CD4+ cells are represented. Similar results were obtained on CD8+ cells. Histograms are representative of two experiments. In the bar graph, the % of proliferating cells is the difference between the percentage of proliferating cells after stimulation by CD3/28 and the percentage of proliferating cells at the same time without stimulation. Black bars represent experiments with necrostatin-1 to block necroptosis.

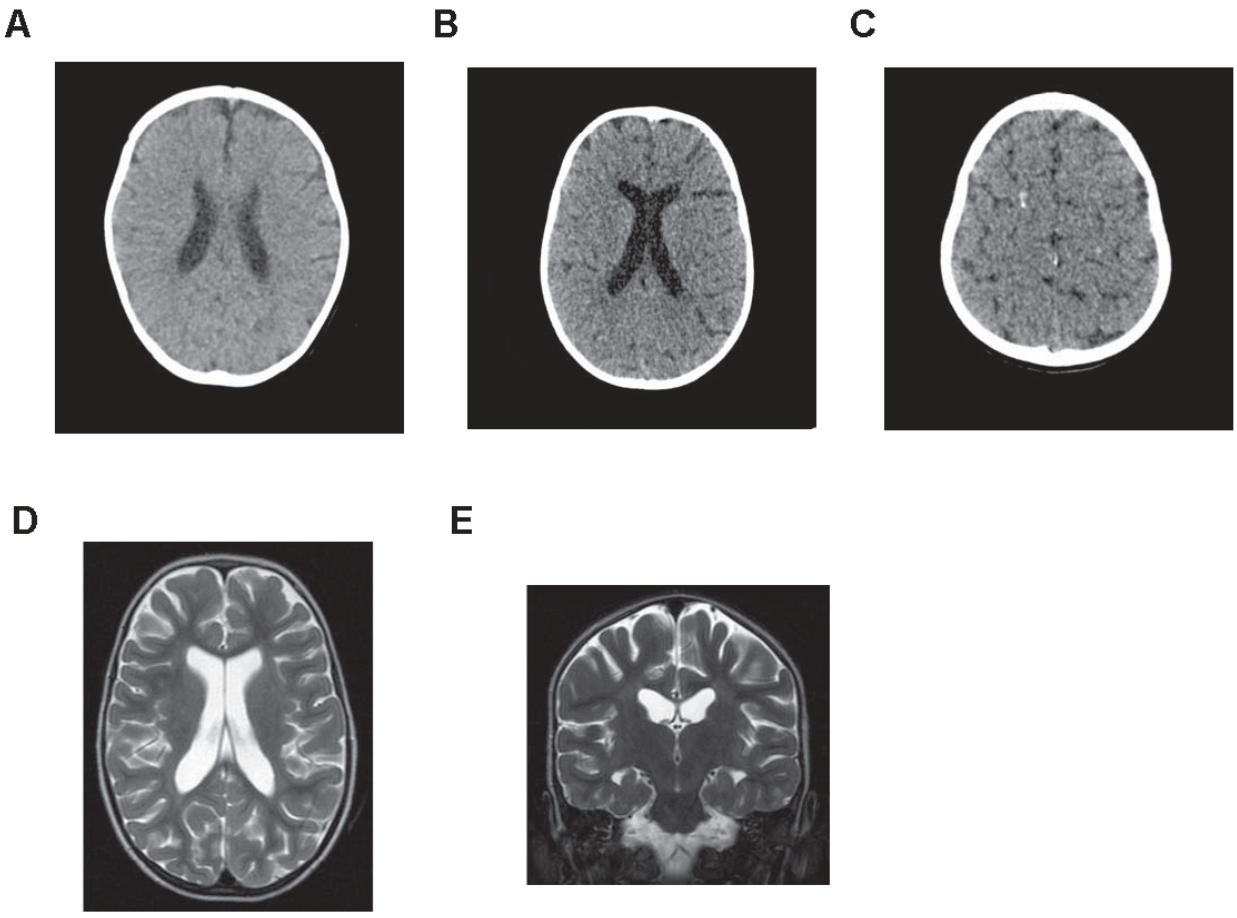

Figure S4. Cranial Imaging in P4 at 5 and 29 Months

(A) Axial CT at 5 months of age. (B-C) Axial CT at 29 months. (B) shows slightly more ex vacuo dilatation of the ventricles and prominent sulci. (C) shows a calcified area in the right cortex and a smaller spot on the left which were not present in previous scans. (D-E) T2-weighted MRI at 29 months. ((D), axial view; (E), coronal view) showing mild global atrophy in keeping with CT appearances. These progressive changes are indicative of an active process.

Table S1. Lymphocyte Subsets and Serum Immunoglobulins

Subject	P4 (2 years)	P4 (13 months[a])	P3 (8 months[a])	P2 (9 months)	Normal range (2 years)
Total lymphocytes (cells/uL)	10116	10259	11905	12670	2000 - 8000
CD3+ (T cells) (% lymphocytes)	7330 (73)	7719 (75)	8446 (71)	9124 (72)	900 – 4500
CD16+CD56+ (NK cells) (% lymphocytes)	581 (6)	553 (5)	1292 (11)	760 (6)	100 – 1000
CD19+ (B cells) (% lymphocytes)	2072 (21)	1858 (18)	1957 (17)	2281 (18)	200 – 2100
Phenotype of CD3+					
CD4 (% total lymphs)	5023 (50)	5536 (54)	6454 (53)	6589 (52)	500 – 2400
CD8 (% total lymphs)	1556 (15)	1520 (15)	1824 (15)	2534 (20)	200 – 1600
CD4/CD8 ratio	3.23	3.64	3.54	2.6	0.9 – 2.9
CD4-CD8-TCRαβ+ (% T cells)	7.8	5			<2.5
activated T cells (% T cells) (HLADR+)	30	15	12		
CD4+ naïve (% T cells) (CD45RA/CD27)	1906 (26)	2856 (37)			
CD4- naïve (% T cells)	1539 (21)	1853 (24)			
Phenotype of B cells					
CD27-IgM+IgD+ (naïve B cells)	81	91			
CD27+IgM+IgD+ (memory B cells)	2	3			
CD27+IgM-IgD- (class switched)	<1	<1			
Serum immunoglobulins (g/L)					
IgG	8.2	10.7	7.72	5.15	4.9 – 16.1
IgA	0.45	0.32	0.47	0.33	0.4 – 2.0
IgM	0.32	0.22	0.56	0.63	0.5 – 2.0
Vaccine-specific immunoglobulins					**(protective)**
Tetanus (IU/ml)	0.74	0.4	2.19		>0.1
Haemophilus influenzae (mg/L)	0.5	>9.0	>9.0		>1.0
Pneumococcal serotype-specific (mg/L):					
4		10.75	1.14		>0.35
6B		4.75	0.67		>0.35
9V		24.95	0.85		>0.35
14		0.86	1.19		>0.35
18C		16.62	0.87		>0.35
19F		1.17	2.42		>0.35
23F		20.48	1.38		>0.35

[a] Serum immunoglobulins in P3 were assessed at 5 months of age; pneumococcal subtype-specific responses were obtained one month after the last dose of conjugate vaccine, aged 3.5 yrs (P3) or 13 months (P4)

Table S2. Whole-Exome-Sequencing Results

Total number of reads		24,989,074
Uniquely Mapped reads		17,816,126
Total variants called		23,146
Substitutions	Total (novel[a])	21,224 (1796)
	Synonymous	9,936
	Nonsense	71
	Missense	8,121
	Splice-site	37
	Non-coding RNAs	58
Indels		1,922
dbSNP rate[b]		91.54%
dbSNP concordance[b]		99.71%
chromosome 11 region	Total[c] (novel[a])	67 (8)
	Synonymous (novel[a])	26 (1)
	Nonsense (novel[a])	0 (0)
	Missense (novel[a])	15 (1)
	Splice-site (novel[a])	0 (0)
	Indels	0
Chromosome 18 region	Total[c] (novel[a])	14 (0)
	Synonymous (novel[a])	6 (0)
	Nonsense (novel[a])	0 (0)
	Missense (novel[a])	8 (0)
	Splice-site (novel[a])	0 (0)
	Indels	0

[a]Number of variants not found in dbSNP129, 1000 Genomes and our in-house database of 70 exomes.
[b]dbSNP rate and concordance are based on dbSNP129.
[c]Homozygous variants.