Supplementary Table 1

Supplementary Table 1: Phenotypic information on patients with missense mutations affecting the N- and C-terminal extended region of the NF1 GRD and evaluation of the pathogenicity of the missense variants

mutation - DNA	Mutation - Protein	Patient ID#	Proband	Age Group	Gender	NIH Criteria	CALMs	Freckling	Lisch	cNFs & sdNFs	PNF	Spinal NF	OPG	Osseous Lesion	Development	Family History	Comments	ESP, EGAC, ESP, EGAC, 1000G	Human Genome Mutation Database (Cardiff)	LOVD	SIFT	MutTaster	PolyPhen	Grantham Distance	Interpretation Pathogenicity	Functional assay
N-terminal extended region of the NF1-GRD																										
c.3604G>T	p.Ala1202Ser	UAB-R9745	Y	0 - ≤8 years	F	+	>5 irregular borders	LA	n	n	n	?	?	n	normal	RS	also found in the father who carries only 1 very small CAL	EA: 0.02%	absent	absent	tolerated (0.22)	Disease Causing (p:1.0)	benign (0.346)	99	class 1 (benign)	normal interaction
c.3611G>T	p.Arg1204Leu	UAB-R7357	Y	9 - ≤18 years	F	-	>5	n	n	n	n	?	no by MRI	frontal bossing	ADD, LD, speech delay	familial CALMs	pineal cyst	absent	absent	absent	deleterious (0.02)	Disease Causing (p:1.0)	probably damaging (0.992)	102	class 3 (variant of uncertain clinical significance)	disrupted interaction
c.3611G>T	p.Arg1204Leu	UAB-R3347	Y	0 - ≤8 years	F	-	>5	n	n	n	n	?	?	n	normal	RS										
c.3611G>T	p.Arg1204Leu	UAB-R07828		≥36 years	M	-	>5	n	n	n	n	NS	?	n	normal	F										
c.3611G>T	p.Arg1204Leu	UAB-R0782	Y	9 - ≤18 years	F	+	>5	BA&I	?	NS	NS	NS			NS	F	Noonan phenotype: short stature, hypertelorism, webbed neck									
c.3611G>T	p.Arg1204Leu	UAB-R3164	Y	9 - ≤18 years	M	+	>5	LI	?	NS					ADD, LD	RS										
c.3610C>G	p.Arg1204Gly	UAB-R5354R	Y	27 - ≤35 years	F	-	>5	n	?	n	n	?	?	n	LD	F		absent	present (Krkljus, 1998)	3x; called pathogenic by curator	deleterious (0.01)	Disease Causing (p:1.0)	probably damaging (0.992)	125	class 4 (likely pathogenic)	normal interaction
c.3610C>G	p.Arg1204Gly	UAB-R5354	Y	0 - ≤8 years	M	-	1-5	RI&LA	?	n	n	?	?	relative macrocephaly	LD, delayed early motor and speech milestones	F										
c.3610C>G	p.Arg1204Gly	UAB-R9937	Y	0 - ≤8 years	M	-	>5	n	?	NS	NS	NS	NS	NS		PrS										
c.3610C>G	p.Arg1204Gly	UAB-R6191	Y	9 - ≤18 years	M	+	>5	BA&I	?	1 cNF	NS	?	no by MRI	scoliosis	?	F	father also CALs									
c.3610C>G	p.Arg1204Gly	UAB-R7021	Y	9 - ≤18 years	F	+	>5	BA&I	B	n	n	?	?	n	LD	PrS										
c.3610C>G	p.Arg1204Gly	UAB-R0684	Y	0 - ≤8 years	M	-	>5	n	n	n	n	?	?	n	NS	PrS	possible Noonan (low set ears)									
c.3610C>G	p.Arg1204Gly	UAB-R3947	Y	9 - ≤18 years	F	+	>5	BA	?	n	suspected on R knee	?	?	n	abnormal LD, autism, PDD	PrS										
c.3610C>G	p.Arg1204Gly	UAB-R583	Y	≥36 years	F	+	>5	NS	?	yes	n	thoracic MRI: several areas of density wi spinal cord; extra axial cyst in posterior fossa	NS	NS		unknown										
c.3610C>G	p.Arg1204Gly	UAB-R0690	Y	27 - ≤35 years	F	+	>5	BA	n	n	n	?	?	n	normal	F	her 8-yo daughter has >6 CALMS and a LD, no mutation									
c.3610C>G	p.Arg1204Gly	UAB-R2353	Y	19 to ≤26 years	M	+	>5	BI	?	2-6 sdNFs	n	n	no by MRI	pectus excavatum	LD	unknown	possible Noonan									
c.3610C>G	p.Arg1204Gly	UAB-R0512	Y	9 - ≤18 years	M	+	>5	NS	B						normal	unknown	pulmonic stenosis									
c.3610C>G	p.Arg1204Gly	UAB-R12252	Y	19 to ≤26 years	M	+	>5	n	n	1 sd NF	n	C6-C7	n	n		unknown										
c.3610C>G	p.Arg1204Gly	UAB-R3631	Y	9 - ≤18 years	M	+	>5	BA&I	?					short stature	DD	unknown										
c.3610C>T	p.Arg1204Trp	UAB-R8035R1		0 - ≤8 years	M	+	>5	n	?	n	n	?	?	n	NS	F		absent	present (Ars, 2000)	absent	deleterious (0.03)	Disease Causing (p:1.0)	probably damaging (1)	101	class 3 (variant of uncertain clinical significance)	disrupted interaction
c.3610C>T	p.Arg1204Trp	UAB-R8035R2		0 - ≤8 years	F	+	>5	BA	n	n	n	?	?	n	normal	F										
c.3610C>T	p.Arg1204Trp	UAB-R8035	Y	≥36 years	F	+	>5	1R1 RA	n	n	n	?	?	n	normal	F										
c.3610C>T	p.Arg1204Trp	UAB-R7214	Y	0 - ≤8 years	M	-	>5	n	?					winging of scapulae	gross motor issues; speech therapy, macrocephalic	unknown	Noonan-like features; downslanting palpebral fissures, broad nasal bridge, bulbous nasal tip,									
c.3623T>G	p.Leu1208Trp	UAB-R2671R		27 - ≤35 years	F	+	>5	2 faint	?	n	n	?	?	scoliosis	normal	F	melanoma on the back									
c.3623T>G	p.Leu1208Trp	UAB-R2671	Y	0 - ≤8 years	F	-	>5	n	?	n	n	?	?	n	normal	F										
c.3623T>G	p.Leu1208Trp	UAB-R7785	Y	0 - ≤8 years	M	-	>5	n	n	n	n	?	?	n	hyperactivity, LD	unknown	Noonan phenotype present: low set ears, midface hypoplasia, hypertelotism	absent	absent	absent	deleterious (0.0)	Disease Causing (p:1.0)	probably damaging (0.999)	61	class 3 (variant of uncertain clinical significance)	disrupted interaction
c.3632T>G	p.Leu1211Arg	UAB-R7694R2		27 - ≤35 years	M	+	>5	n	?	2-6 cNFs	n	?	no by MRI	n	had remedial reading when younger	F		absent	absent	absent	deleterious (0.01)	Disease Causing (p:1.0)	probably damaging (0.997)	102	class 3 (variant of uncertain clinical significance)	normal interaction
c.[3632T>G];[2728G>C]	p.[Leu1211Arg];[Gly910Arg]	UAB-R7694	Y	0 - ≤8 years	F	-	3-4	n	n	n	n	NS	?	macrocephaly	abnormal development	F	proband carries two NF1 variants in trans; p.Leu1211Arg was found in the affected father; p.Gly910Arg was found in the unaffected mother and is a likely benign rare variant									
c.3639_3641delAAT	p.Met1215del	UAB-R0757	Y	0 - ≤8 years	F	+	>5	LI	?			NS	NS	NS	NS	RS		absent	present (Lee, 2006)	5x; called likely pathogenic by curator	NA	NA	NA	NA	class 4 (likely pathogenic)	disrupted interaction
c.3639_3641delAAT	p.Met1215del	UAB-R8947	Y	0 - ≤8 years	F	+	>5	1-2 RA	?	1-2 possible cNFs	NS	NS	NS	NS	NS	RS	possible Juvenile Xanthogranuloma									
c.3639_3641delAAT	p.Met1215del	UAB-R3694	Y	9 - ≤18 years	F	+	>5	BI&A	?	n	n	?	?	n	LD	RS										
c.3639_3641delAAT	p.Met1215del	UAB-R3912	Y	≥36 years	M	+	>5	BA&I	B	>100 cNFs and 6-99 sdNFs	l. leg	NS	NS	NS	NS	F										
c.3650A>G	p.Asp1217Gly	UAB-R3763R		27 - ≤35 years	M	+	>5	A	?	NS	NS	NS	NS	NS	NS	F		absent	absent	absent	tolerated (0.1)	Disease Causing (p:1.0)	probably damaging (0.996)	94	class 4 (likely pathogenic)	disrupted interaction
c.3650A>G	p.Asp1217Gly	UAB-R3763	Y	0 - ≤8 years	M	+	>5	LI&A	?	s	NS	NS	NS	NS	abnormal development	F										
c.3650A>G	p.Asp1217Gly	UAB-R3532R2		0 - ≤8 years	M	+	>5	I	?	NS	n	?	no by MRI	n	hyperactivity	F	short stature, Noonan??									
c.3650A>G	p.Asp1217Gly	UAB-R3532R1		19 to ≤26 years	F	-	3	BA&I	?	NS	NS	NS	NS	NS	NS	F										
c.3650A>G	p.Asp1217Gly	UAB-R3532	Y	0 - ≤8 years	M	-	>5	n	n	n	n	?	?	n	normal	F										
c.3650A>G	p.Asp1217Gly	UAB-R2381R6		9 - ≤18 years	F	+	>5	BA&I	?	n	n	?	?	NS	normal	F										
c.3650A>G	p.Asp1217Gly	UAB-R2381R5		9 - ≤18 years	F	+	>5	BA&I	?	n	n	?	?	NS	normal	F										
c.3650A>G	p.Asp1217Gly	UAB-R2381R2		0 - ≤8 years	F	+	>5	n	?	NS	NS	NS	NS	NS	NS	F										
c.3650A>G	p.Asp1217Gly	UAB-R2381R3		27 - ≤35 years	M	+	>5	BA&I	?	n	n	?	NS	mild assymmetry spine	normal	F										
c.3650A>G	p.Asp1217Gly	UAB-R2381R1		27 - ≤35 years	F	+	>5	BA&I	?	7 subdermal	n	NS	?	mild scoliosis	normal	F										
c.3650A>G	p.Asp1217Gly	UAB-R2381	Y	27 - ≤35 years	F	+	>5	bil ax	?	1 subdermal	n	?	?	n	?	F										
c.3650A>G	p.Asp1217Gly	UAB-R9707	Y	0 - ≤8 years	M	-	>5	n	n	n	n	?	?	n	normal	S	proven to be de novo; but father with 3 large CALs and mild scoliosis									
c.3650A>G	p.Asp1217Gly	UAB-R1971	Y	9 - ≤18 years	F	+	>5	BA&I	?	NS	NS	NS	NS	NS	NS	unknown										
C-terminal extended region of the NF1-GRD																										
c.4469T>C/c.4532T>C	p.Leu1490Pro/p.Leu1511Pro	UAB-R6344R		≥36 years	M	+	>5	NS	?	6-99 cNFs	NS		?	n	normal	F		absent	present: NF??	2x; called pathogenic by curator	deleterious (0.01)	Disease Causing (p:1.0)	probably damaging (1)	98	class 4 (likely pathogenic)	disrupted interaction
c.4469T>C/c.4532T>C	p.Leu1490Pro/p.Leu1511Pro	UAB-R6344	Y	≥36 years	M	+	>5	n	n	n	n	?	present by MRI, symptomatic, chiasm	n	normal	F										
c.4469T>C/c.4532T>C	p.Leu1490Pro/p.Leu1511Pro	UAB-R4702	Y	9 - ≤18 years	F	-	>5	n		n	n	?	?	scoliosis	normal	unknown										
c.4481A>G; c.4544A>G	p.Gln1494Arg/p.Gln1515Arg	UAB-R5602	Y	9 - ≤18 years	M	-	>5	n	n	n	n	?	?		normal	PrS		absent	present (Van Minkelen, 2014)	1x; called pathogenic by curator	deleterious (0.02)	Disease Causing (p:1.0)	probably damaging (0.989)	43	class 4 (likely pathogenic)	normal interaction
c.4493G>A; 4556G>A	p.Gly1498Glu/p.Gly1519Glu	UAB-R5527R1		27 - ≤35 years	M	+	>5	n	?	2 sdNFs	n	?			abnormal development, ADD, hyperactivity, LD, speech delay	F	intermittent pericarditis	absent	present (Ars, 2003)	absent	tolerated (0.23)	Disease Causing (p:1.0)	probably damaging (0.99)	98	class 3 (variant of uncertain clinical significance)	normal interaction
c.4493G>A; 4556G>A	p.Gly1498Glu/p.Gly1519Glu	UAB-R5527	Y	0 - ≤8 years	M	+	>5	n	n	n	?	?	?	n	normal	F										
c.4493G>A; 4556G>A	p.Gly1498Glu/p.Gly1519Glu	UAB-R6312	Y	0 - ≤8 years	F	+	>5	yes	n	n	n	?	?	n	learning disability	unknown	VSD, Noonan possibe (short stature)									
c.4493G>A; 4556G>A	p.Gly1498Glu/p.Gly1519Glu	UAB-R3811	Y	0 - ≤8 years	M	-	>5	?	?	NS	NS	NS	NS	NS												
c.4493G>A; 4556G>A	p.Gly1498Glu/p.Gly1519Glu	UAB-R314	Y	0 - ≤8 years	F	+	>5	yes	?	NS	NS	NS	NS	NS	mild speech delay	familial CALMs										

Legend:
+ present; - absent; ? Unknown; NS not specified; M male; F female;
+ NIH criteria fulfilled; - NIH criteria not fulfilled
l: luguinal; A axillary; L left; R right; B bilateral
cNF cutaneous neurofibroma; sdNF subdermal neurofibroma; LD learning difficulties;
RS: reported as sporadic, parents not available for testing; PrS: proven de novo by testing both unaffected parents, but no paternity/maternity testing performed; F familial
Classification: 5: pathogenic; 4: likely pathogenic; 3: variant of unknown significance; 2: likely benign; 1: benign

Supplementary Table 2: Phenotypic information on patients with missense mutations in the EVH1 domain of *SPRED1*

mutation - DNA	mutation - protein	patient ID#	Age (yr) / Gender	CALM	freckling	Macrocephaly	Learning Disabilities	Other Findings	Family History	Reference	ESP; ExAC; 1000G	Evolutionary Conservation	SIFT	Mutation Taster	Polyphen	Grantham Distance	Pathogenicity
c.26A>T	p.Asp9Val	UAB-S103	5.5/M	>6	-	?	?	-	?	Brems et al. 2012; dbSNP	ESP: 0.02%; ExAC: 0.14-1.32% (Latino and South Asian)	Moderately conserved amino acid	deleterious (score 0,01)	disease causing (p=1)	benign (0,0002)	125	class 1 (benign)
c.26A>T	p.Asp9Val	UAB-SPR941	36/F	>6	bilateral axillary and inguinal freckling	?	?	proven pathogenic NF1 mutation, OPG, neurofibromas, SPRED1 mutation in unaffected brother	familial	newly described							
c.26A>T	p.Asp9Val	UAB-SPR881	10/F	8	right axillary	?	?	-	?	newly described							
c.26A>T	p.Asp9Val	UAB-SPR491	37/F	-	-	?	?	diffuse histological confirmed gastric neurofibromas, bone cysts on wrist and scoliosis	?	newly described							
c.30C>A	p.Asn10Lys	UAB-S84	10/M	>6	axillary and inguinal	?	+	likely pathogenic missense mutation in NF1 (K263E), also in affected father both NF1 and SPRED1 mutations present	familial	Brems et al. 2012 dbSNP	Eur Am: 0.02%; Eur non-Finnish: 0.16%	Moderately conserved amino acid	deleterious (score 0)	disease causing (p:0.99)	benign (0,042)	94	class 1 (benign)
c.30C>A	p.Asn10Lys	UAB-S133	2/M	2 CALMs R upper quadrant, 7 cm hyperpigmented region on R neck	4-5 freckles R neck, R axillary freckling			segmental distribution of pigmentary abnormalities, abnormal development	?	newly described							
c.71G>A	p.Arg24Gln	BISC-I2	u/F	2	-	-	-	-	familial	newly described	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	43	class 4 (likely pathogenic)
c.71G>A	p.Arg24Gln	BISC-II3	u/M	3	-	>97th	-	-	familial	newly described							
c.71G>A	p.Arg24Gln	BICS-III1	new born/F	8	-	97th	-	scoliosis	familial	newly described							
c.71G>A	p.Arg24Gln	BISC-III3	9 months/M	9	axillar	50th	-	-	familial	newly described							
c.71G>A	p.Arg24Gln	UAB-S52	20/F	>6	bilateral axillary	?	?	left congenital ventricular aneurism	?	Spencer et al. 2011							
c.71G>A	p.Arg24Gln	UAB-S119	37/M	>6	bilateral axillary	?	?	2-6 skin nodules	?								
c.71G>A	p.Arg24Gln	UAB-S432	8/F	>6	bilateral	-	-	maternal family history of CALM	familial	newly described							
c.88G>A	p.Gly30Arg	UAB-S46	?	?	?	?	?	?	?	Spencer et al. 2011	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	125	class 4 (likely pathogenic)
c.92G>T	p.Trp31Leu	UAB-S129	5.5/M	8	?	?	?	mild thoracic scoliosis, abnormal development with heart murmur, possible Noonan phenotype with short stature and midface hypoplasia	familial	newly described	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	61	class 4 (likely pathogenic)
c.93G>T	p.Trp31Cys	Denayer-family 3: II	15/M	>5	-	?	language delay, ADHD	hypertelorism, downslanting palpebral fissues, ptosis, low implanted postriorly rotated ears, low posterior hairline, mild pterygium colli, pectus carinatum/excavatum, motor delay	de novo	Denayer et al. 2011	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	215	class 5 (pathogenic)
c.93G>T	p.Trp31Cys	Net-16	M	multiple	?	?	?	-	?								
c.93G>T	p.Trp31Cys	UAB-SPR061	F	>6	bilateral axillary and inguinal		+	-	?	newly described here							
c.124G>A	p.Val42Ile	UAB-S68	8/u	8-10	-	?	?	spinal tumors and bilateral vestibular schwannomas, pathogenic NF2 mut	?	Spencer et al. 2011, dbSNP	ExAC SAS:0.4%; ESP AA:0.07%	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 0,999)	29	class 1 (benign)
c.124G>A	p.Val42Ile	UAB-S77	9 months/M	>6	-	?	?		?								
c.124G>A	p.Val42Ile	UAB-SPR451	8/F	>6	-	?	?	variant also present in unaffected mother	familial	newly described here							
c.131T>A	p.Val44Asp	Spurlock-family 6	60-68/M	+	+	98th	?	phenotype of affected son and 2 grandchildren of proband described with same mutation	familial	Spurlock et al. 2009	absent	Highly conserved amino acid, up to Zebrafish	deleterious (score 0)	disease causing (p=1)	probably damaging (score 0,999)	152	class 4 (likely pathogenic)
c.184G>C	p.Gly62Arg	Utah-1	3/F	+	-	+	?	-	familial	newly described here	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	125	class 4 (likely pathogenic)
c.221G>T	p.Cys74Phe	UAB-S5/SB4	?	?	?	?	?	segmental distribution of pigmentary abnormalities	?	Messiaen et al. 2009	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	205	class 3 (variant of uncertain clinical significance)
c.239T>G	p.Leu80Arg	UAB-S78	11/M	>6	-	-	-	-	sporadic	Spencer et al. 2011	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 0,995)	102	class 3 (variant of uncertain clinical significance)
c.263C>A	p.Thr88Lys	Fe-7	7/M	multiple	axillary and inguinal	?	?	mutation also present in affected mother	familial	newly described here - LOVD-SPRED1 - Pasmant Paris	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0.02)	disease causing (p=1)	probably damaging (score 1)	78	class 4 (likely pathogenic)
c.274T>C	p.Trp92Arg	UAB-S127	3/M	>6	?	?	-	also found in the proband's mother with 1 hyperpigmented region on the abdomen	familial	newly described here	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	101	class 4 (likely pathogenic)
c.299G>A	p.Gly100Asp	UAB-S104	62/F	>6	present	?	?	?	?	Brems et al. 2012	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	94	class 4 (likely pathogenic)
c.305C>T	p.Thr102Met	UAB-S83-I1	26/F	>6	-	-	+	short stature, headache	familial	Spencer et al. 2011	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	81	class 3 (variant of uncertain clinical significance)
c.305C>T	p.Thr102Met	UAB-S83-II1	11 months/F	>6	-	-	-		familial								
c.305C>A	p.Thr102Lys	UAB-S64	0,75/M	>6	-	-	-	?	sporadic	Spencer et al. 2011	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	78	class 4 (likely pathogenic)
c.305C>A	p.Thr102Lys	UAB-S105	2/M	>6	-	-	-	?	sporadic	Brems et al. 2012							
c.305C>G	p.Thr102Arg	UAB-S24	17/M	>6	+	?	?	large right temporal venous anomaly in brain, progressive dystonia of unexplained etiology	familial	Messiaen et al. 2009	absent	Highly conserved amino acid, up to Fruitfly	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	71	class 4 (likely pathogenic)
c.347T>A	p.Ile116Asn	UAB-S110-I1	35/M	>6	+	?	-		familial	newly described here	absent	Highly conserved amino acid, up to Zebrafish	deleterious (score 0)	disease causing (p=1)	probably damaging (score 1)	149	class 3 (variant of uncertain clinical significance)
c.347T>A	p.Ile116Asn	UAB-S110-II1	21 months/M	>6	-	?	?		familial	newly described here							

Legend:
M male
F female
y years
u unknown
- not present
+ present
pathogenicity: score according to Richards et al., Genetics in Medicine, 17:405-424, 2015.
1= benign
2= likely benign
3= uncertain significance
4= likely pathogenic
5= pathogenic