Supporting Information

Supp. Methods. Summary of methodology and research approach.

Supp. Table S1. In *silico* prediction of pathogenicity and *in vivo* splicing analysis of all possible *NF1* missense variants affecting p.Met1149.

Supp. Table S2. In *silico* prediction of pathogenicity and *in vivo* splicing analysis of all possible *NF1* missense variants affecting p.Arg1276.

Supp. Table S3. In *silico* prediction of pathogenicity and *in vivo* splicing analysis of all possible *NF1* missense variants affecting p.Lys1423.

Supp. Table S4. Clinical details for individuals with *NF1* splice variants mimicking missense variants at p.Lys1423.

Supp. Table S5. The classification of *NF1* missense variants affecting p.Met1149, p.Arg1276 and p.Lys1423 reported in the studied group according to the ACMG recommendations.

Supp. Table S6. Clinical details for 70 individuals with one of four different amino substitutions at *NF1* p.Met1149 from 51 different families.

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Supp. Table S13. Spectrum of clinical features in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Lys1423.

Supp. Table S14. Spinal neurofibromas in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276.

Supp. Table S15. Spinal neurofibromas in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Lys1423.

Supp. Table S16. Plexiform neurofibromas in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Lys1423.

Supp. Table S17. Plexiform neurofibromas in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276.

Supp. Table S18. Comparison of clinical features of the cohort of individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276 and p.Lys1423 with the cohort of the individuals carrying one out of the 18 most frequent recurrent pathogenic nonsense variants observed in the University of Alabama at Birmingham (UAB) database.

Supp. Table S19. Clinical characterization of individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276 who did not fulfill the current NIH diagnostic criteria, based on physical features, not taking family history into account.

Supp. Table S20. Noonan-like features in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276.

Supp. Table S21. Noonan-like features in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Lys1423.

Supp. Table S22. Cardiovascular abnormalities in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276.

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Supp. Table S24. List of all adjusted *P* values from Table 1 after applying the Benjamini-Hochberg correction for multiple testing with false discovery rates (FDR) at 0.05 and 0.01.

Supp. Table S25. List of all adjusted *P* values from Table 2 after applying the Benjamini-Hochberg correction for multiple testing with false discovery rates (FDR) at 0.05 and 0.01.

Supp. Table S26. List of all adjusted *P* values from Table 3 after applying the Benjamini-Hochberg correction for multiple testing with false discovery rates (FDR) at 0.05 and 0.01.

Supp. Table S27. List of all adjusted *P* values from Supporting Information Table S18 after applying the Benjamini-Hochberg correction for multiple testing with false discovery rates (FDR) at 0.05 and 0.01.

Supp. Table S28. Skeletal abnormalities in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Met1149, p.Arg1276 and p.Lys1423.

Supp. Table S29. Comparison of clinical features of the cohorts of individuals heterozygous for pathogenic *NF1* missense variants affecting p.Met1149, p.Arg1276 and p.Lys1423 referred for molecular *NF1* genetic testing to the Medical Genomics Laboratory at the University of Alabama at Birmingham (UAB) and to the collaborating institutions in Europe (EUR).

Supp. Table S30. List of all adjusted *P* values from Supporting Information Table S29 after applying the Benjamini-Hochberg correction for multiple testing with false discovery rates (FDR) at 0.05 and 0.01.

Supp. Table S31. The prevalence of pulmonic stenosis in the studied cohorts and in individuals heterozygous for pathogenic *NF1* missense variants affecting codons 1809 and 844-848, p.Met992del, the cohort of the individuals carrying one out of the 18 most frequent recurrent pathogenic nonsense variants observed in the University of Alabama at Birmingham (UAB) database and in the general NF1 population.

Supp. Table S32. The overall prevalence of cardiac/cardiovascular abnormalities, excluding hypertension as the only feature, in the studied cohorts and in individuals heterozygous for pathogenic *NF1* missense variants affecting codons 1809 and 844-848, p.Met992del, the cohort of the individuals carrying one out of the 18 most frequent recurrent pathogenic nonsense variants observed in the University of Alabama at Birmingham (UAB) database and in the general NF1 population.

Supp. Figure S1. The classification of *NF1* missense variants affecting p.Met1149, p.Arg1276 and p.Lys1423: comparison of the classification reported in publicly available disease databases (HGMD, LOVD and ClinVar) with the current study (as of December 2018).

Supp. Figure S2. Pedigrees of the familial cases heterozygous for pathogenic *NF1* missense variants affecting p.Met1149 in the current study.

Supp. Figure S3. Pedigrees of the familial cases heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276 in the current study.

Supp. Figure S4. Pedigrees of the familial cases heterozygous for pathogenic *NF1* missense variants affecting p.Lys1423 in the current study.

Supp. Figure S5. Coronal T2-weighted STIR MR images of the cervical (A), lumbosacral (B) and dorsal (C) spine showing bilateral giant and fairly confluent plexiform neurofibromas of all paraspinal nerves and plexuses in individual EUR-R22-F. Note the intraspinal tumor origin in all the spinal foramina which appear widening without extension in the spinal canal. Referred by Marica Eoli, MD (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy).

Supp. Methods. Summary of methodology and research approach.

The collaborating institutions included the following centers: Molecular Genetics Unit, Casa Sollievo della Sofferenza Hospital, IRCSS, San Giovanni Rotondo, Italy (11 probands and 3 relatives), University Hospital, Ghent, Belgium (10 probands and 2 relatives), Carlo Besta Neurological Institute, IRCSS, Milan, Italy (10 probands and 1 relative), University of Leuven, Belgium (8 probands), Medical University of Innsbruck, Austria (5 probands and 2 relatives), University of Padova, Italy (6 probands) and Hospital Universitario Ramón y Cajal, IRYCIS and CIBERER, Madrid, Spain (5 probands).

Comprehensive *NF1* molecular screening using an RNA-based approach complemented by DNA-dosage analysis was performed in the Medical Genomics Laboratory at UAB as previously described (Messiaen et al., 2000; Messiaen et al., 2012) with LRG_214 and NM_000267.3 used as the reference sequences. For exon numbering we used the NCBI numbering, followed by the historical numbering originally developed by the NF1 community in square brackets (Messiaen et al., 2012). To predict the pathogenic effect of the missense variants described herein, we used the same approach as previously reported (Rojnueangnit et al., 2015; Koczkowska et al., 2018) according to the ACMG recommendations (Richards et al., 2015).

Clinical data were collected as previously described (Rojnueangnit et al., 2015; Koczkowska et al., 2018; Koczkowska et al., 2019) using a detailed standardized phenotypic checklist form at the time of genetic testing and re-verified by referring physicians for accuracy and/or updating, when available. As no magnetic resonance imaging (MRI) results were available for most individuals without clinical signs/suspicions for spinal neurofibromas or optic pathway gliomas (OPGs), presence of internal neurofibromas and asymptomatic OPGs could not be excluded in all cases. An individual was classified as having a Noonan-like phenotype when at least two of the following features were present: short stature, dysmorphic facies (low set ears, hypertelorism, downslanting palpebral fissures, midface hypoplasia, ptosis and/or webbed neck), pectus and/or cardiac abnormalities. Genetic testing of Noonan-related disorder gene(s) was performed in individuals presenting with Noonan-like features (NS; MIM# 163950) (Supporting Information Tables S6, S8, S10, S12 and S20-S21). Height and head circumference at a given age were converted to percentiles at PC≤3 for short stature and PC≥ 98 for macrocephaly as previously reported (Rojnueangnit et al., 2015; Koczkowska et al., 2018; Koczkowska et al., 2019). To establish genotype-phenotype correlations, we used the same approach as previously described (Rojnueangnit et al., 2015; Koczkowska et al., 2018; Koczkowska

et al., 2019) by comparing the phenotypes in the studied cohorts with the cohorts of individuals heterozygous for pathogenic *NF1* missense variants affecting codons 1809 and 844-848 (Nyström et al., 2009; Ekvall et al., 2014; Pinna et al., 2015; Rojnueangnit et al., 2015; Santoro et al., 2015; Koczkowska et al., 2018), the *NF1* p.Met992del (Upadhyaya et al., 2007; Koczkowska et al., 2019) and cohorts of individuals with "classic" NF1 phenotype (Huson et al., 1988; Huson et al., 1989a; Huson et al., 1989b; Listernick et al., 1994; Friedman et al., 1997; Cnossen et al., 1998; McGaughran et al., 1999; Thakkar et al., 1999; Lin et al., 2000; Blazo et al., 2004; Khosrotehrani et al., 2005; Plotkin et al., 2012; Blanchard et al., 2016). Additionally, we compared the phenotypes in the studied cohorts with the cohort of individuals carrying one out of the 18 most recurrent nonsense variants identified in the UAB database.

Supp. Table S1. In *silico* prediction of pathogenicity and *in vivo* splicing analysis of all possible *NF1* missense variants affecting p.Met1149.

Variant	Protein	p.Met1149 positive probands	gnomAD (123,136)	EVS (6,503)	1000 G	HGMD [A]	LOVD (4,602) [A]	ClinVar (3,941) [A]	Grantham distance	SIFT	PolyPhen	CADD	REVEL	SSF/MaxEntScan/ NNSplice	RNA observed	Other evidence
c.3445A>C	p.Met1149Leu	0/51	0	0	0	0	0	0	15	Tolerated (0.6)	Benign (0.257)	25.3	0.362	No change	N/A	-
c.3445A>G	p.Met1149Val	38/51	<0.01%	0	0	Present (DM)	5 (P)	2 (P/LP)	21	Deleterious (0.04)	Benign (0.452)	24.3	0.875	Creates a novel splice acceptor sequence: from WT (-) / (-) / (-) to MUT 79.2 / 5.8 / 0.2	Normal missense	Proven de novo (UAB-R9123, UAB-R9563, UAB-R1324, UAB-R7725, UAB-R58801FN.203); segregation in multiple families (UAB-R8931, UAB-R7911, UAB-R0292, UAB-R9123, UAB-R0154, UAB-R7654, UAB-R5694, UAB-R7885, UAB-R61001FN.201)
c.3445A>T	p.Met1149Leu	1/51	0	0	0	0	0	0	15	Tolerated (0.6)	Benign (0.257)	25.5	0.362	No change	Normal missense	observed in a single proband (UAB-R1495)
c.3446T>A	p.Met1149Lys	0/51	0	0	0	0	0	0	95	Deleterious (0)	Possibly damaging (0.814)	28.5	0.838	Creates a novel splice acceptor sequence: from WT (-) / (-) / (-) to MUT (-) / 3.0 / (-)	N/A	-
c.3446T>C	p.Met1149Thr	6/51	0	0	0	0	0	0	81	Deleterious (0.01)	Possibly damaging (0.814)	25.1	0.827	No change	Normal missense	Proven de novo (UAB-R4191, UAB-R1667); segregation in family (UAB-R3656)
c.3446T>G	p.Met1149Arg	0/51	0	0	0	0	0	1 [B] (VUS)	91	Deleterious (0)	Possibly damaging (0.914)	26.7	0.826	Creates a novel splice acceptor sequence: from WT (-) / (-) / (-) to MUT (-) / 3.9 / (-) Creates a novel splice donor sequence: from WT (-) / (-) / (-) to MUT 72.3 / 1.8 / 0.1	N/A	-
c.3447G>A	p.Met1149Ile	4/51	0	0	0	Present (DM)	1 (VUS)	3 [C] (P/LP)	10	Tolerated (0.06)	Possibly damaging (0.656)	27.7	0.628	No change	Normal missense	Segregation in multiple families (UAB-R2347, UAB-R5855, UAB-R2474)
c.3447G>C	p.Met1149Ile	1/51	0	0	0	0	0	1 (P)	10	Tolerated (0.06)	Possibly damaging (0.656)	26.7	0.429	No change	N/A	Segregation in family (UAB-R86801FN.103)
c.3447G>T	p.Met1149Ile	1/51	0	0	0	0	0	1 (P)	10	Tolerated (0.06)	Possibly damaging (0.656)	27	0.627	No change	Normal missense	Segregation in family (UAB-R7741)

[A] The variants' classification presented in round brackets is as reported by the original author(s) (as of December 2018); [B] The variant was reported in a single individual with hereditary cancer-predisposing syndrome, not NF1; [C] Including 2 individuals with NF1 (classified as *pathogenic* and *likely pathogenic* in each individual) and 1 individual with an unknown condition (the condition type was not provided by the original author) with a variant classification as *likely pathogenic*.

Abbreviations - gnomAD: the **Genom**e **A**ggregation **D**atabase; **EVS**: **E**xome **V**ariant **S**erver; **1000 G**: **1000 G**enomes Project; **HGMD**: **H**uman **G**ene **M**utation **D**atabase; **LOVD**: **L**eiden **O**pen **V**ariation **D**atabase; **SIFT**: **S**orting **I**ntolerant **F**rom **T**olerant; **CADD**: **C**ombined **A**nnotation **D**ependent **D**epletion; **REVEL**: **R**are **E**xome **V**ariant **E**nsemble **L**earner (Ioannidis et al., 2016); **SSF**: **S**plice**S**ite**F**inder-like; **VUS**: **v**ariant of **u**ncertain **s**ignificance; **P**: **p**athogenic; **LP**: **l**ikely **p**athogenic; **DM**: disease causing; **N/A**: **n**ot **a**pplicable.

Supp. Table S2. In *silico* prediction of pathogenicity and *in vivo* splicing analysis of all possible *NF1* missense variants affecting p.Arg1276.

Variant	Protein	p.Arg1276 positive probands [A]	gnomAD (123,136)	EVS (6,503)	1000 G	HGMD [B]	LOVD (4,602) [B]	ClinVar (3,941) [B]	Grantham distance	SIFT	PolyPhen	CADD	REVEL	SSF/MaxEntScan/ NNSplice	RNA observed	Other evidence
c.3826C>G	p.Arg1276Gly	14/100	0	0	0	Present (DM)	4 (P)	3 [C] (LP/NP)	125	Deleterious (0)	Probably damaging (0.992)	33	0.878	No change	Normal missense	Proven de novo (UAB-R1175, UAB-R8312, UAB-R8876, UAB-R3396; EUR-R9); segregation in family (UAB-R1175; UAB-R37411FN.204)
c.3827G>A [D]	p.Arg1276Gln	80/100	<0.01% [E]	0	0	Present (DM)	14 [F] (P/LP)	7 [G] (P/NP)	43	Deleterious (0)	Probably damaging (0.997)	34	0.887	No change	Normal missense	Proven de novo (UAB-R0082, UAB-R3605, UAB-R8805, UAB-R6515, UAB-R0306, UAB-R8666, UAB-R221011FN.101; EUR-R27); segregation in multiple families (UAB-R0522, UAB-R7733, UAB-R8484, UAB-R0215, UAB-R1256, UAB-R6328: UAB-R743; UAB-R834; EUR-R21; EUR-R22; EUR-R28)
c.3827G>C	p.Arg1276Pro	3/100	0	0	0	Present (DM)	0	3 (P)	103	Deleterious (0)	Probably damaging (0.998)	34	0.903	Increases strength of a cryptic exonic splice acceptor sequence from WT 76.2 / (-) / (-) to MUT 87.1 / 6.1 / 0.9	Normal missense	-
c.3827G>T	p.Arg1276Leu	3/100	0	0	0	0	0	0	102	Deleterious (0)	Probably damaging (0.992)	34	0.918	Increases strength of a cryptic exonic splice acceptor sequence from WT 76.2 / (-) / (-) to MUT 80.9 / 6.4 / 0.9	Normal missense	Segregation in families (UAB-R3393, UAB-R8304)

[A] Excluding one proband (UAB-R5166) heterozygous for the *NF1* c.3826_3827delinsGA (p.Arg1276Glu; see details in Supp. Information Table S10); [B] The variants' classification presented in round brackets is as reported by the original author(s) (as of December 2018); [C] Conflicting interpretations of pathogenicity, including *likely pathogenic* in 1 individual with NF1 and in 1 individual with an unknown condition (no details provided by the author); for the third individual no variant's classification nor condition type was provided; [D] A single individual (UAB-R9572) carried a second *NF1* variant c.8002G>A (p.Val2668Met); as the individual's parents were not available for follow-up and for inheritance verification, according to the ACMG guidelines c.8002G>A (p.Val2668Met) is classified as VUS (see details in Supp. Information Table S10); [E] Variant c.3827G>A (p.Arg1276Gln) is absent in gnomAD database when specifically looking only in a cohort of individuals with no neurological features and non-cancer phenotype; [F] Including 13 individuals with the *NF1* p.Arg126Gln variant classified as *pathogenic* and 1 individual as *likely pathogenic*; [G] Conflicting interpretations of pathogenicity, including *pathogenic* in 5 individuals (3 with NF1 and 2 with an unknown condition type); for 2 additional individuals (1 with NF1 and 1 with an unknown condition type) no variant classification was provided.

Abbreviations - gnomAD: the **G**e**nom**e **A**ggregation **D**atabase; **EVS**: **E**xome **V**ariant **S**erver; **1000 G**: **1000 G**enomes Project; **HGMD**: **H**uman **G**ene **M**utation **D**atabase; **LOVD**: **L**eiden **O**pen **V**ariation **D**atabase; **SIFT**: **S**orting **I**ntolerant **F**rom **T**olerant; **CADD**: **C**ombined **A**nnotation **D**ependent **D**epletion; **REVEL**: **R**are **E**xome **V**ariant **E**nsemble **L**earner (Ioannidis et al., 2016); **SSF**: **S**plice**S**ite**F**inder-like; **VUS**: **v**ariant of **u**ncertain **s**ignificance; **P**: **p**athogenic; **LP**: **l**ikely **p**athogenic; **DM**: disease causing; **NP**: **n**ot **p**rovided.

Supp. Table S3. In *silico* prediction of pathogenicity and *in vivo* splicing analysis of all possible *NF1* missense variants affecting p.Lys1423.

Variant	Protein	p.Lys1423 positive probands	gnomAD (123,136)	EVS (6,503)	1000 G	HGMD [A]	LOVD (4,602) [A]	ClinVar (3,941) [A]	Grantham distance	SIFT	PolyPhen	CADD	REVEL	SSF/MaxEntScan/ NNSplice	RNA observed	Other evidence
c.4267A>C	p.Lys1423Gln	7/87	0	0	0	Present (DM?)	2 (VUS)	0	53	Deleterious (0)	Probably damaging (0.989)	26.4	0.919	Increases strength of the splice donor sequence from WT 83.2 / 6.4 / 0.4 to MUT 83.5 / 6.6 / 0.4	Normal missense	Proven de novo (UAB-R9805)
c.4267A>G [B]	p.Lys1423Glu	77/87	0	0	0	Present (DM)	20 [C] (P/LP)	7 [D] (P/VUS)	56	Deleterious (0)	Probably damaging (0.974)	28.1	0.905	Decreases strength of the splice donor sequence from WT 83.2 / 6.4 / 0.4 to MUT 80.4 / 5.4 / 0.3	Normal missense	Proven de novo (UAB-R2741, UAB-R1442, UAB-R5313, UAB-R5973, UAB-R5344, UAB-R1185; EUR-R55); segregation in family (UAB-R4031, UAB-R5651, EUR-R39, EUR-R48, EUR-R54)
c.4268A>C	p.Lys1423Thr	2/87	0	0	0	0	0	0	78	Deleterious (0)	Probably damaging (0.989)	27	0.932	Decreases strength of the splice donor sequence from WT 83.2 / 6.4 / 0.4 to MUT 74.3 / 5.4 / 0.3	Low level of r.4111_4269del (p.Val1371_Lys1423del) predominant missense [E]	-
c.4268A>G	p.Lys1423Arg	0/87 [F]	0	0	0	Present (DM)	1 (P)	0	26	Deleterious (0)	Probably damaging (0.974)	27.2	0.849	Decreases strength of the splice donor sequence from WT 83.2 / 6.4 / 0.4 to MUT 74.2 / 2.6 / 0.2	r.4111_4269del (p.Val1371_Lys1423del) predominant splicing	-
c.4268A>T	p.Lys1423Met	1/87	0	0	0	Present (DM)	0	0	95	Deleterious (0)	Probably damaging (0.999)	28.1	0.931	Decreases strength of the splice donor sequence from WT 83.2 / 6.4 / 0.4 to MUT 74.7 / 3.5 / 0.1	Low level of r.4111_4269del (p.Val1371_Lys1423del) predominant missense [E]	Proven de novo (UAB-R1753)
c.4269G>C	p.Lys1423Asn	0/87 [F]	0	0	0	Present (DM)	0	2 [G] (P/LP)	94	Deleterious (0)	Probably damaging (0.989)	29.4	0.859	Eliminates a splice donor sequence: from WT 83.2 / 6.4 / 0.4 to MUT (-) / (-) / (-)	r.4111_4269del (p.Val1371_Lys1423del) predominant splicing	-
c.4269G>T	p.Lys1423Asn	0/87 [F]	0	0	0	Present (DM)	1 [H] (P)	0	94	Deleterious (0)	Probably damaging (0.989)	29.9	0.859	Decreases strength of a splice donor sequence: from WT 83.2 / 6.4 / 0.4 to MUT 70.6 / (-) / (-)	r.4111_4269del (p.Val1371_Lys1423del) predominant splicing	-

[A] The variants' classification presented in round brackets is as reported by the original author(s) (as of December 2018); [B] Two individuals (UAB-R1185 and EUR-R55) carried a second *NF1* variant: c.8042A>T (p.Tyr2681Phe) and c.584A>G (p.Lys195Arg), respectively; according to the ACMG guidelines both variants are classified as likely benign (see details in Supp. Table S12); [C] Including 18 individuals with the *NF1* p.Lys1423Glu variant classified as *pathogenic* and in 2 individuals as *likely pathogenic*; [D] Including 6 individuals (4 individuals with NF1, 1 individual with hereditary cancer-predisposing syndrome and 1 with an unknown condition type) with the *NF1* p.Lys1423Glu variant classified as *pathogenic* and 1 individual with NF1 as *variant of uncertain significance*; [E] c.4268A>C and c.4268A>T caused very low missplicing and the major effect was still missense and therefore individuals heterozygous for these two variants were included in the genotype-phenotype analysis, except for c.4268A>T that is considered as "*likely pathogenic*" variant, not pathogenic according to the current ACMG recommendations;[1] [F] In the current study, we identified presence of c.4268A>G, c.4269G>C and c.4269G>T in 4, 3 and 2 individuals, respectively (see phenotypic details in Supp. Table S4); all variants reported in the UAB cohort lead exclusively to skipping of exon 32 [24] during *NF1* mRNA splicing, r.4111_4269del (p.Val1371_Lys1423del), thus they are not included in Supp. Table S12 as these alterations should be classified as splicing variants *mimicking* a missense variant, not bona fide missense variants; as no RNA-based approach was applied in the collaborating centers, the missplicing effect could not be confirmed in individuals EUR-R56, EUR-R57, EUR-R58 and EUR-R59; however, it is expected to be identical to what was observed in the UAB cohort; [G] The *NF1* p.Lys1423Asn missense variant was classified as *pathogenic* in 1 individual with NF1 and as *likely pathogenic* in 1 individual with hereditary cancer-predisposing syndrome; [H] The variant was reported in the LOVD database as a missense *pathogenic variant*, however, no RNA-based analysis was performed in this individual (only DNA-dosage analysis). **Abbreviations - gnomAD**: the **G**e**nom**e **A**ggregation **D**atabase; **EVS**: **E**xome **V**ariant **S**erver; **1000 G**: **1000 G**enomes Project; **HGMD**: **H**uman **G**ene **M**utation **D**atabase; **LOVD**: **L**eiden **O**pen **V**ariation **D**atabase; **SIFT**: **S**orting **I**ntolerant **F**rom **T**olerant; **CADD**: **C**ombined **A**nnotation **D**ependent **D**epletion; **REVEL**: **R**are **E**xome **V**ariant **E**nsemble **L**earner (Ioannidis et al., 2016); **SSF**: **S**plice**S**ite**F**inder-like; **VUS**: **v**ariant of **u**ncertain **s**ignificance; **P**: **p**athogenic; **LP**: **l**ikely **p**athogenic; **DM**: disease causing; **DM?**: likely disease causing.

Supp. Table S4. Clinical details for individuals with *NF1* splice variants mimicking missense variants at p.Lys1423.

Supp. Table S4 is included as a separate Excel file.

[A] For individuals with one asterisk (*) the standardized phenotypic checklist forms were not re-verified/updated by the referring physicians, the data are based on the originally submitted forms; individuals with two asterisks (**) had incomplete phenotypic checklist forms.

[B] **UAB**: **U**niversity of **A**labama at **B**irmingham (5 probands); **EUR**: individual referred by the **Eur**opean collaborators (4 probands), Molecular Genetics Unit, Casa Sollievo della Sofferenza Hospital, IRCSS, San Giovanni Rotondo, Italy (3 probands) and University of Leuven, Belgium (1 proband).

[C] **RS**: **r**eportedly **s**poradic; **NS**: **n**ot **s**pecified

[D] Exact age was used to calculate Height and Head Circumferences (HC), but provided as age-groups in Supporting Information Table S4: 0-24 months; 2-4 years; 5-8 years; 9-13 years; 14-18 years; 19-26 years; >26 years.

[E] **F**: **f**emale; **M**: **m**ale

[F] **W**: **W**hite

[G] An individual was classified as having a Noonan-like phenotype if at least two of the following features were present: short stature (SS), low set ears (LSE), hypertelorism (HTL), midface hypoplasia (MH), webbed neck (WN), pectus abnormality (PA) or pulmonic stenosis (PS).

[H] Height percentiles for Hispanic individuals were provided in square brackets to indicate that they were excluded from the data analysis on frequency of short or normal stature due to the lack of ethnic-specific growth charts.

[I] Comprehensive alphabetical list of all genes mentioned in Supporting Information Table S4: *NF1* (MIM# 613113) and *SPRED1* (MIM# 609291).

Abbreviations:

NS: **n**ot **s**pecified, i.e. no value provided on the phenotypic checklist; **UN**: **un**kown; **bil**: **bil**ateral; **gr**: **gr**oin; **ax**: **ax**illary; **CALMs**: **c**afé-**a**u-**l**ait **m**acule**s** **OPG**: **o**ptic **p**athway **g**lioma; **N, UN**: **n**o symptomatic OPG or symptomatic spinal neurofibroma, **un**known if any asymptomatic OPG or asymptomatic spinal neurofibromas are present; **N, MRI**: **n**o symptomatic and asymptomatic OPG or spinal neurofibroma detected by **MRI**; **HTL**: **h**yper**t**e**l**orism; **WN**: short/**w**ebbed **n**eck; **MH**: **m**idface **h**ypoplasia; **LSE**: **l**ow **s**et **e**ars; **LPH**: **l**ow **p**osterior **h**airline; **SS**: **s**hort **s**tature; **PA**: **p**ectus **a**bnormality; **PT**: **pt**osis; **PS**: **p**ulmonic **s**tenosis; **DPF**: **d**ownslanting **p**alpebral **f**issures; **HC**: **h**ead **c**ircumference; **SD**: **s**peech **d**elay; **MRI**: **m**agnetic **r**esonance **i**maging..

Supp. Table S5. The classification of *NF1* missense variants affecting p.Met1149, p.Arg1276 and p.Lys1423 reported in the studied group according to the ACMG recommendations.

Variant	Protein	PS1	PS2 [A]	PS3	PS4	PM1 [B]	PM2	PM3	PM4	PM5 [C]	PM6 [D]	PP1 [E]	PP2	PP3	PP4 [F]	PP5 [G]	Variant classification [H]
c.3445A>G	p.Met1149Val	N/A	**YES**	?	**YES**	?	NO	N/A	N/A	N/A	N/A	**YES**	?	NO	**YES**	**YES** [1-3]	**Pathogenic** → **Pathogenic**
c.3445A>T	p.Met1149Leu	N/A	N/A	?	NO	?	**YES**	N/A	N/A	**YES**	N/A	N/A	?	NO	**YES** [I]	NO	**?** → **VUS**
c.3446T>C	p.Met1149Thr	N/A	N/A	?	**YES**	?	**YES**	N/A	N/A	**YES**	**YES**	N/A	?	**YES**	**YES**	NO	**?** → **Pathogenic**
c.3447G>A	p.Met1149Ile	N/A	N/A	?	**YES**	?	**YES**	N/A	N/A	**YES**	N/A	**YES**	?	NO	**YES** [I]	**YES** [1]	**Pathogenic** → **Pathogenic**
c.3447G>C	p.Met1149Ile	**YES**	N/A	?	NO	?	**YES**	N/A	N/A	**YES**	N/A	**YES** [J]	?	NO	**YES** [I]	NO	**?** → **Pathogenic**
c.3447G>T	p.Met1149Ile	**YES**	N/A	?	NO	?	**YES**	N/A	N/A	**YES**	N/A	N/A	?	NO	**YES**	**YES** [3]	**Pathogenic** → **Pathogenic**
c.3826C>G	p.Arg1276Gly	N/A	**YES**	**YES** [K]	**YES**	**YES**	**YES**	N/A	N/A	N/A	N/A	N/A	?	**YES**	**YES**	**YES** [1,2]	**Pathogenic** → **Pathogenic**
c.3827G>A	p.Arg1276Gln	N/A	**YES**	**YES** [K]	**YES**	**YES**	NO	N/A	N/A	N/A	N/A	**YES**	?	**YES**	**YES**	**YES** [1-3]	**Pathogenic** → **Pathogenic**
c.3827G>C	p.Arg1276Pro	N/A	N/A	**YES** [K]	**YES**	**YES**	**YES**	N/A	N/A	N/A	N/A	N/A	?	**YES**	**YES**	**YES** [1,3]	**Pathogenic** → **Pathogenic**
c.3827G>T	p.Arg1276Leu	N/A	N/A	?	**YES**	**YES**	**YES**	N/A	N/A	**YES**	N/A	N/A	?	**YES**	NO	NO	**?** → **Pathogenic**
c.4267A>C	p.Lys1423Gln	N/A	N/A	**YES** [K]	**YES**	**YES**	**YES**	N/A	N/A	**YES**	**YES**	N/A	?	**YES**	**YES**	NO	**VUS** → **Pathogenic**
c.4267A>G	p.Lys1423Glu	N/A	**YES**	**YES** [K]	**YES**	**YES**	**YES**	N/A	N/A	N/A	N/A	N/A	?	**YES**	**YES**	**YES** [1-3]	**Pathogenic** → **Pathogenic**
c.4268A>C	p.Lys1423Thr	N/A	N/A	?	**YES**	**YES**	**YES**	N/A	N/A	**YES**	N/A	N/A	?	**YES**	NO	NO	**?** → **Pathogenic**
c.4268A>G	p.Lys1423Arg	N/A [L]	**YES**	**YES** [K]	**YES**	**YES**	**YES**	N/A	**YES**	N/A	N/A	N/A	?	**YES**	**YES**	**YES** [1,2]	**Pathogenic** → **Pathogenic**
c.4268A>T	p.Lys1423Met	N/A	N/A	?	NO	**YES**	**YES**	N/A	N/A	**YES**	**YES**	N/A	?	**YES**	NO	**YES** [1]	**Pathogenic** → **Likely pathogenic**
c.4269G>C	p.Lys1423Asn	N/A [L]	N/A	**YES** [K]	**YES**	**YES**	**YES**	N/A	**YES**	N/A	N/A	N/A	?	**YES**	**YES**	**YES** [1,3]	**Pathogenic** → **Pathogenic**
c.4269G>T	p.Lys1423Asn	N/A [L]	**YES**	**YES** [K]	**YES**	**YES**	**YES**	N/A	**YES**	N/A	N/A	N/A	?	**YES**	**YES**	**YES** [1,2]	**Pathogenic** → **Pathogenic**

PS1: same amino acid change as a previously established pathogenic variant regardless of nucleotide change; **PS2**: proven *de novo* (both maternity and paternity confirmed); **PS3**: well-established functional studies; **PS4:** the prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls; **PM1**: located in a mutational hot spot and/or in critical functional domain; **PM2**: absent from controls; **PM3**: for recessive disorders, detected in *trans* with a pathogenic variant; **PM4**: protein length changes due to in-frame or stop-loss variants; **PM5**: novel missense change at amino acid residue where a different pathogenic missense change has been seen before; **PM6**: assumed *de novo*, but without confirmation of paternity and maternity; **PP1**: co-segregation with disease in multiple affected family members; **PP2**: missense variant in a gene that has a low rate of benign missense variation; **PP3**: multiple lines of computational evidence support a deleterious effect on the gene or gene product; **PP4**: individual's phenotype or family history is highly specific for a disease; **PP5**: reputable source reports variant as pathogenic; **N/A**: not applicable; **YES**: the criterion has been fulfilled; **NO**: the criterion has not been fulfilled; **?**: due to the lack of evidence, it is difficult to establish whether the criterion has been fulfilled or not

[A] based on the data from LOVD database (only individuals confirmed as *de novo* and identified in the Netherlands where biological relationships were confirmed as part of the *de novo* assessment have been taken into account); [B] only GRD domain (amino acids 1,217-1,511) has been proved to be critical to protein function, thus this evidence could be considered as moderate evidence of pathogenicity for the *NF1* missense variants affecting codons 1276 and 1423; [C] variants classified as *pathogenic* in the publicly available disease databases (HGMD, LOVD, ClinVar) have been taken into account; [D] only individuals confirmed as *de novo* at UAB have been taken into account, except for variants fulfilling the PS2 criterion (p.Met1149Val, p.Arg1276Gly, p.Arg1276Gln, p.Lys1423Glu, p.Lys1423Arg and p.Lys1423Asn); [E] segregation with at least three affected family members needed to fulfill this criterion; [F] at least one individual with "classic" NF1 phenotype, including neurofibroma(s) and/or Lisch nodules was considered required to fulfill this criterion; [G] based on the variant's classification as *pathogenic* reported in HGMD (1), LOVD (2) and/or ClinVar (3) databases (details in Supporting Information Tables S1-S3); [H] prior variants classification as reported in the publicly available disease databases (HGMD, LOVD, ClinVar) and *a posteriori* classification based on our study and following the ACMG recommendations (Richards et al., 2015); [I] individuals, carrying c.3445A>T (p.Met1149Leu), c.3447G>A (p.Met1149Ile) or c.3447G>C (p.Met1149Ile), presented only with pigmentary manifestations, that is, >5 CALMs and/or skinfold freckles; however, RASopathy NGS panel or *SPRED1* testing was negative (see details in Supporting Information Table S6); [J] segregation with only two affected family members, however, RASopathy NGS panel was negative, thus the proband's and the proband's mother phenotypes were highly specific for NF1 (see details in Supporting Information Table S6); [K] based on Poullet et al. (1994), Klose et al. (1998) and/or Scheffzek and Welti (2012); [L] the variants c.4268A>G, c.4269G>C and c.4269G>T lead to skipping of exon 32 [24] during *NF1* mRNA splicing, r.4111_4269del (p.Val1371_Lys1423del) in all individuals referred to the UAB (see details in Supporting Information Table S4), thus these alterations should be classified as splicing variants *mimicking* a missense variant, not bona fide missense variants.

To classify variant as pathogenic the following criteria need to be fulfilled: ≥2 strong (PS1-PS4) OR 1 strong (PS1-PS4) and ≥3 moderate (PM1-PM6) OR 1 strong (PS1-PS4) and 2 moderate (PM1-PM6) and ≥2 supporting (PP1-PP5) OR 1 strong (PS1-PS4) and 1 moderate (PM1-PM6) and ≥4 supporting (PP1-PP5).

To classify variant as likely pathogenic the following criteria need to be fulfilled: 1 strong (PS1-PS4) and 1-2 moderate (PM1-PM6) OR 1 strong (PS1-PS4) and ≥2 supporting (PP1-PP5) OR ≥3 moderate (PM1-PM6) OR 2 moderate (PM1-PM6) and ≥2 supporting (PP1-PP5) OR 1 moderate (PM1-PM6) and ≥4 supporting (PP1-PP5).

Variant should be classified as **Variant of Uncertain Significance (VUS)** if other criteria are unmet or the criteria for benign and pathogenic are contradictory.

Supp. Table S6. Clinical details for 70 individuals with one of four different amino substitutions at *NF1* p.Met1149 from 51 different families.

Supp. Table S6 is included as a separate Excel file.

[A] For individuals with one asterisk (*) the standardized phenotypic checklist forms were not re-verified/updated by the referring physicians, the data are based on the originally submitted forms; individuals with two asterisks (**) had incomplete phenotypic checklist forms.

[B] **UAB**: **U**niversity of **Al**abama at **B**irmingham (45 probands and 19 relatives); **EUR**: individuals referred by the **Eur**opean collaborators (6 probands), including Molecular Genetics Unit, Casa Sollievo della Sofferenza Hospital, IRCSS, San Giovanni Rotondo, Italy (3 probands), University of Padova, Italy (1 proband), University Hospital, Ghent, Belgium (1 proband) and University of Leuven, Belgium (1 proband).

[C] **F**: **f**amilial; **PrS**: **pr**oven **s**poradic; **RS**: **r**eportedly **s**poradic; **NS**: **n**ot **s**pecified; **UN**: **un**known

[D] Exact age was used to calculate Height and Head Circumferences (HC), but provided as age-groups in Supporting Information Table S6: 0-24 months; 2-4 years; 5-8 years; 9-13 years; 14-18 years; 19-26 years; >26 years.

[E] **F**: **f**emale; **M**: **m**ale.

[F] **W**: **W**hite; **His**: **His**panic; **AA**: **A**frican **A**merican; **As**: **As**ian; **O: O**ther; **NA**: **N**ative **A**merican.

[G] An individual was classified as having Noonan-like phenotype if at least two of the following features were present: short stature (SS), low set ears (LSE), hypertelorism (HTL), midface hypoplasia (MH), webbed neck (WN), ptosis (PT), downslanting palpebral fissures (DPF), pectus abnormality (PA) or pulmonic stenosis (PS).

[H] Height percentiles for Hispanic and Asian individuals were provided in square brackets to indicate that they were excluded from the data analysis on frequency of short or normal stature due to the lack of ethnic-specific growth charts.

[I] Individuals with ADD/ADHD, but normal development were still classified as normal.

[J] RASopathy panel performed at UAB included 17 genes: *NF1, SPRED1, PTPN11, PPP1CB, BRAF, CBL, HRAS, KRAS, NRAS, MAP2K1, MAP2K2, RAF1, RIT1, RASA2, SHOC2, SOS1* and *SOS2*.

[K] Comprehensive alphabetical list of all genes mentioned in Supporting Information Table S6: *ACTC1* (MIM# 102540), *A2ML1* (MIM# 610627), *BRAF* (MIM# 164757), *CAV3* (MIM# 601253), *CBL* (MIM# 165360), *GLA* (MIM# 300644), *HRAS* (MIM# 190020), *KAT6B* (MIM# 605880), *KRAS* (MIM# 190070), *LAMP2* (MIM# 309060), *MAP2K1* (MIM# 176872), *MAP2K2* (MIM# 601263), *MT-TG* (MIM# 590035), *MT-TI* (MIM# 590045), *MT-TK* (MIM# 590060), *MT-TQ* (MIM# 590030), *MYBPC3* (MIM# 600958), *MYH7* (MIM# 160760), *MYL2* (MIM# 160781), *MYL3* (MIM# 160790), *NF1* (MIM# 613113), *NRAS* (MIM# 164790), *PPP1CB (MIM# 600590)*, *PRKAG2* (MIM# 602743), *PTPN11* (MIM# 176876), *RAF1* (MIM# 164760), *RASA2* (MIM# 601589), *RIT1* (MIM# 609591), *RRAS* (MIM# 165090), *SHOC2* (MIM# 602775), *SOS1* (MIM# 182530), *SOS2* (MIM# 601247), *SPRED1* (MIM# 609291), *TNNC1* (MIM# 191040), *TNNI3* (MIM# 191044*)*, *TNNT2* (MIM# 191045), *TPM1* (MIM# 191010) and *TTR* (MIM# 176300).

[L] UAB-R1495, carrying c.3445A>T (p.Met1149Leu), was excluded from the genotype-phenotype study as the interpretation of this specific variant is "VUS" according to the ACMG recommendations (see details in Supporting Information Table S5).

Abbreviations: NS: **n**ot **s**pecified, i.e. no value provided on the phenotypic checklist; **UN**: **un**kown**; CALMs**: **c**afé-**a**u-**l**ait **m**acule**s**; **bil**: **bil**ateral; **gr**: **gr**oin; **ax**: **ax**illary; **sub**: **sub**mammary; **OPG**: **o**ptic **p**athway **g**lioma; **N, UN**: **n**o symptomatic OPG or symptomatic spinal neurofibroma, **un**known if any asymptomatic OPG or asymptomatic spinal neurofibromas are present; **N, MRI**: **n**o symptomatic and asymptomatic OPG or spinal neurofibroma detected by **MRI**; **HTL**: **h**yper**t**e**l**orism; **WN**: short/**w**ebbed **n**eck; **MH**: **m**idface **h**ypoplasia; **LSE**: **l**ow **s**et **e**ars; **LPH**: **l**ow **p**osterior **h**airline; **SS**: **s**hort **s**tature; **PA**: **p**ectus **a**bnormality; **PT**: **pt**osis; **PS**: **p**ulmonic **s**tenosis; **DPF**: **d**ownslanting **p**alpebral **f**issures; **HC**: **h**ead **c**ircumference; **ADD**: **a**ttention **d**eficit **d**isorder; **ADHD**: **a**ttention **d**eficit **h**yperactivity **d**isorder; **ID**: **i**ntellectual **d**isability; **LD**: **l**earning **d**isability; **SD**: **s**peech **d**elay; **F**: **f**ather; **M**: **m**other; **S**: **s**ibling; **C**: **c**hild; **FSIQ**: **F**ull **S**cale **I**ntelligence **Q**uotient; **MRI**: **m**agnetic **r**esonance **i**maging; **NGS**: **n**ext **g**eneration **s**equencing; **VUS**: **v**ariant of **u**ncertain **s**ignificance.

Supp. Table S7. Spectrum of clinical features in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Met1149.

Pathogenic variant	**c.3445A>G (p.Met1149Val)**			**c.3446T>C (p.Met1149Thr)**			**c.3447G>A (p.Met1149Ile) c.3447G>C (p.Met1149Ile) c.3447G>T (p.Met1149Ile)**			**All pathogenic variants**			**Total**
Pathogenic variant positive individuals (Proband:Relative)	49 (38:11)			7 (6:1)			13 (6:7)			69 (50:19)			69 (50:19)
Age group, years	**≤8**	**9-18**	**≥19**	**≤8**	**9-18**	**≥19**	**≤8**	**9-18**	**≥19**	**≤8**	**9-18**	**≥19**	**all ages**
Total	16	18	15	1	1	5	6	2	5	23	21	25	69
Proband:Relative	15:1	15:3	8:7	1:0	1:0	4:1	2:4	1:1	3:2	18:5	17:4	15:10	50:19
Male: Female	9:7	9:9	6:9	0:1	0:1	2:3	6:0	1:1	3:2	15:8	10:11	11:14	36:33
Fulfilling the NIH criteria if the family history is taken into account	10/16	17/18	10/15	0/1	0/1	3/5	5/6	2/2	4/5	15/23	19/21	17/25	51/69
Fulfilling the NIH criteria if solely taking the physical signs into account	8/16	15/18	9/15	0/1	0/1	1/5	3/6	1/2	3/5	11/23	16/21	13/25	40/69
>5 CALMs	15/16	17/18	13/15	1/1	1/1	3/5	5/6	2/2	5/5	21/23	20/21	21/25	62/69
Skinfold freckling	8/14	16/18	9/15	0/1	0/1	0/3	3/6	1/2	3/5	11/21	17/21	12/23	40/65
Lisch nodules	0/11	1/14	0/7	0/1	0/1	1/3	0/3	0/2	1/2	0/15	1/17	2/12	3/44
Skeletal abnormalities [A]	1/14	4/17	6/12	0/1	0/1	1/4	2/6	1/2	0/4	3/21	5/20	7/20	15/61
Plexiform neurofibromas	0/13	0/16	0/14	0/1	0/1	0/5	0/6	0/2	0/4	0/20	0/19	0/23	0/62
Cutaneous neurofibromas [B]	0/15	0/17	3/15	0/1	0/1	0/5	0/6	0/2	0/4	0/22	0/20	3/24	3/66
Subcutaneous neurofibromas [B]	0/14	1/16	3/14	0/1	0/1	0/5	0/6	0/2	0/3	0/21	1/19	3/22	4/62
Symptomatic spinal neurofibromas	0/12	0/17 [C]	0/13	0/0	0/1	0/5	0/6	0/2	0/3	0/18	0/20	0/21	0/59
Symptomatic OPG [D]	0/12	0/17	0/13	0/1	0/1	0/3	0/5	0/2	0/4	0/18	0/20	0/20	0/58
Asymptomatic OPG [E]	0/4	0/9	0/5	0/1	0/0	0/0	0/1	0/2	0/1	0/6	0/11	0/6	0/23
Other neoplasms [F]	0/11	2/18	2/11	0/1	0/1	0/3	0/5	0/2	2/5	0/17	2/21	4/19	6/57
Cognitive impairment and/or learning disabilities	8/15	11/18	6/13	1/1	0/1	0/5	3/6	2/2	0/5	12/22	13/21	6/23	31/66
Noonan-like phenotype [G]	3/14	5/17	5/14	0/1	0/1	1/4	2/6	1/2	1/3	5/21	6/20	7/21	18/62 [H]
Short stature [I]	1/7	3/14	0/8	0/1	0/0	1/1	0/0	0/1	0/1	1/8	3/15	1/10	5/33
Macrocephaly	7/12	7/14 [J]	1/3	0/1	0/0	1/4	1/5	1/2	1/4	8/18	8/16	3/11	19/45
Pulmonic stenosis	0/12	1/14	0/10	0/1	0/1	1/4	0/6	0/2	0/2	0/19	1/17	1/16	2/52
Cardiovascular abnormalities [K]	1/12	2/14	1/10	0/1	0/1	1/4	0/6	0/2	0/2	1/19	2/17	2/16	5/52

[A]All bone abnormalities included, that is, pectus abnormality (n=8), scoliosis (n=3), marfanoid habitus (n=1), genu valgum (n=1), sphenoid wing dysplasia (n=1), leg length discrepancy (n=1), clinodactyly (n=1) and exostosis (n=1), except for scoliotic attitude ("pseudoscoliosis") observed in two individuals (EUR-R1 and EUR-R2, Supporting Information Table S6). [B] At least two cutaneous/subcutaneous neurofibromas were required to be considered as "positive for the criterion of neurofibromas". [C] A *possible* spinal lesion was reported by MRI at T12 region of spine in a single 18-year-old individual (UAB-R2593, Supporting Information Table S6), however, this lesion requires the differential diagnosis between spinal neurofibroma and cyst. [D] The absence of symptomatic OPGs was determined by ophthalmological examination and/or by MRI. [E] Including only individuals without signs of symptomatic OPGs who underwent MRI examination. [F] All neoplasms, excluding OPGs and neurofibromas, included, that is, lipoma(s) (n=4), benign lesion in the right temporal lobe detected by MRI (n=1) and pilomatrixoma (n=1). [G] An individual was classified as having a Noonan-like phenotype when at least two of the following features were present: short stature, low set ears, hypertelorism, downslanting palpebral fissures, midface hypoplasia, ptosis, webbed neck, pectus abnormality and/or pulmonic stenosis. [H] Excluding five individuals with *possible* Noonan-like features, but further clinical details were not available. [I] As no specific growth curves are available for the Hispanic and Asian populations, Hispanic and Asian individuals were excluded as having short or normal stature. [J] Including a single individual (UAB-R2593) with head circumference PC97 classified as a macrocephaly. [K] All cardiovascular abnormalities included, that is, pulmonic stenosis (n=2), atrial septal defect (n=2), quadricuspid aortic valve (n=1) and hypertrophic cardiomyopathy (n=1).

Supp. Table S8. Noonan-like features in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Met1149.

ID [A]	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Others	Noonan-like phenotype
UAB-R5694-S	c.3445A>G (p.Met1149Val)	F	<2	<6	-	-	MH, LSE, motor delay, SD
UAB-R58801FN.203	c.3445A>G (p.Met1149Val)	PrS	<2	>5	+	hydrocephalus	mild HTL, LPH, a slightly coarse look, macrocephaly
UAB-R6393	c.3445A>G (p.Met1149Val)	UN	5-8	>5	+	-	*possible*: SS, LSE, macrocephaly
EUR-R2	c.3445A>G (p.Met1149Val)	RS	5-8	>5	+	sphenoid wing dysplasia, scoliotic attitude, atrial septal defect	DPF, coarse facial features, bulbous nasal tip, bitemporal narrowing, frontal bossing, high anterior hairline, macrocephaly
UAB-R86801FN.103	c.3447G>C (p.Met1149Ile)	F	5-8	>5	+	-	HTL, PA
UAB-R2347	c.3447G>A (p.Met1149Ile)	F	5-8	>5	-	a history of joint hypermobility, headaches	*possible*: SS, HTL, SD, LD
UAB-R2347-S1	c.3447G>A (p.Met1149Ile)	F	5-8	>5	-	a history of joint hypermobility, clinodactyly	PT, WN, PA, SD, LD
UAB-R8931-S	c.3445A>G (p.Met1149Val)	F	9-13	>5	+	-	mild PT, MH, HTL, LSE, DPF, fine motor delay, LD, macrocephaly
UAB-R7654-S	c.3445A>G (p.Met1149Val)	F	9-13	<6	-	-	HTL, LSE, MH, macrocephaly
UAB-R2347-S2	c.3447G>A (p.Met1149Ile)	F	9-13	>5	-	headaches, musculoskeletal aches and pains	HTL, DPF, PA, SD, LD
UAB-R8931	c.3445A>G (p.Met1149Val)	F	14-18	>5	+	2-6 subcutaneous neurofibromas, epilepsy	SS, PT, MH, HTL, LSE, DPF, bitemporal narrowing, narrow face, dolichocephaly, PA, LD, ADD, developmental delay
UAB-R7654	c.3445A>G (p.Met1149Val)	F	14-18	>5	+	-	HTL, LSE, WN, MH, LD, macrocephaly
UAB-R97601FN.203	c.3445A>G (p.Met1149Val)	UN	14-18	>5	+	marfanoid habitus, pilomatrixoma	*possible* (no details provided), LD
EUR-R1	c.3445A>G (p.Met1149Val)	RS	14-18	>5	+	scoliotic attitude	PT, LSE, SD, LD
UAB-R5694-M	c.3445A>G (p.Met1149Val)	F	19-26	>5	+	-	HTL, LSE, WN, MH, abnormal development, LD, SD
UAB-R8931-M	c.3445A>G (p.Met1149Val)	F	>26	>5	-	-	PT, MH, HTL, LSE, DPF, LD
UAB-R0292-F	c.3445A>G (p.Met1149Val)	UN	>26	>5	+	2-6 cutaneous, subcutaneous and intradermal neurofibromas	*possible* (no details provided), PA, abnormal development, LD
UAB-R7654-M	c.3445A>G (p.Met1149Val)	NS	>26	<6	-	lipomas	HTL, LSE, MH
UAB-R9615	c.3445A>G (p.Met1149Val)	RS	>26	>5	+	-	mild PT, DPF, short neck, wide-set nipples, PA, hypertrophic cardiomyopathy, macrocephaly, LD
UAB-R4138	c.3445A>G (p.Met1149Val)	UN	>26	>5	-	seizure disorder	HTL, PT, LSE, frontal bossing
EUR-R5	c.3446T>C (p.Met1149Thr)	F	>26	>5	-	-	DPF, LSE, LPH, WN
UAB-R86801FN.103-M	c.3447G>C (p.Met1149Ile)	F	>26	>5	+	-	HTL, DPF
UAB-R2347-F	c.3447G>A (p.Met1149Ile)	F	>26	>5	-	lipoma	*possible*: SS, HTL

[A] IDs of individuals with the negative RASopathy NGS panel were bolded (see details in Supporting Information Table S6).

Abbreviations: NS - **n**ot **s**pecified; **UN** - **un**known; **F** - **f**amilial; **RS** - **r**eportedly **s**poradic; **PrS** - **p**roven **s**poradic; **HTL** - **h**yper**t**e**l**orism; **WN** - short/**w**ebbed **n**eck; **MH** - **m**idface **h**ypoplasia; **LSE** - **l**ow **s**et **e**ars; **LPH** - **l**ow **p**osterior **h**airline; **SS** - **s**hort **s**tature; **PA** - **p**ectus **a**bnormality; **PT** - **pt**osis; **PS** - **p**ulmonic **s**tenosis; **DPF** - **d**ownslanting **p**alpebral **f**issures; **ADD** - **a**ttention **d**eficit **d**isorder; **ADHD** - **a**ttention **d**eficit **h**yperactivity **d**isorder; **LD** - **l**earning **d**isability; **SD** - **s**peech **d**elay.

Supp. Table S9. Clinical characterization of individuals heterozygous for pathogenic *NF1* missense variants affecting p.Met1149 who did not fulfill the current NIH diagnostic criteria, based on physical features, not taking family history into account.

ID [A, B]	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Lisch nodules	Neurofibromas	OPG	Specific skeletal abnormalities [C]	NIH diagnostic criteria [D]
UAB-R5694-S	c.3445A>G (p.Met1149Val)	F	<2	<6	-	-	-	N, UN	-	0/6
UAB-R7741-C	c.3447G>T (p.Met1149Ile)	F	<2	-	-	-	-	N, UN	-	0/6
UAB-R83811FN.205	c.3445A>G (p.Met1149Val)	RS	2-4	>5	-	-	-	N, UN	-	1/6
UAB-R4191	c.3446T>C (p.Met1149Thr)	PrS	2-4	>5	-	-	-	N, MRI	-	1/6
UAB-R2347-S1	c.3447G>A (p.Met1149Ile)	F	5-8	>5	-	-	-	N, UN	-	1/6
UAB-R9123-C	c.3445A>G (p.Met1149Val)	F	9-13	>5	-	-	-	N, MRI	-	1/6
UAB-R2347-S2	c.3447G>A (p.Met1149Ile)	F	9-13	>5	-	-	-	N, MRI	-	1/6
UAB-R1667	c.3446T>C (p.Met1149Thr)	PrS	14-18	>5	-	-	-	N, UN	-	1/6
UAB-R56201FN.201	c.3445A>G (p.Met1149Val)	RS	19-26	>5	-	-	-	N, MRI	-	1/6
UAB-R7654-M	c.3445A>G (p.Met1149Val)	NS	>26	<6	-	-	-	N, UN	-	0/6
UAB-R5374	c.3445A>G (p.Met1149Val)	RS	>26	-	-	-	-	N, UN	-	0/6

[A] Of the 29 cases not fulfilling the NIH diagnostic criteria after excluding the family history, 11 had complete clinical information including the ophthalmological results for the presence/absence of Lisch nodules and symptomatic OPGs; [B] IDs of individuals with none of the diagnostic criteria of NF1 fulfilled were bolded; [C] Only skeletal abnormalities with sphenoid dysplasia or thinning of the long bone cortex with/without pseudarthrosis have been taken into account; [D] Only clinical features without the presence/absence of a first-degree relative that meets NIH criteria have been taken into account.

Abbreviations: F - **f**amilial; **PrS** - **pr**oven **s**poradic; **RS** - **r**eportedly **s**poradic; **NS** - **n**ot **s**pecified; **UN** - **un**known; **CALMs** - **c**afé-**a**u-**l**ait **m**acule**s**; **OPG** - **o**ptic **p**athway **g**lioma; **N, UN** - **n**o symptomatic OPG, **un**known if any asymptomatic OPG is present; **N, MRI** - **n**o symptomatic and asymptomatic OPG detected by **MRI**.

Supp. Table S10. Clinical details for 119 individuals with one of five different amino substitutions at *NF1* p.Arg1276 from 101 different families.

Supp. Table S10 is included as a separate Excel file.

[A] For individuals with one asterisk (*) the standardized phenotypic checklist forms were not re-verified/updated by the referring physicians, the data are based on the originally submitted forms; individuals with two asterisks (**) had incomplete phenotypic checklist forms.

[B] **UAB**: **U**niversity of **A**labama at **B**irmingham (75 probands and 15 relatives); **EUR**: individuals referred by the **Eur**opean collaborators (26 probands and 3 relatives), including Medical University of Innsbruck, Austria (2 probands and 1 relative), Hospital Universitario Ramón y Cajal, IRYCIS and CIBERER, Madrid, Spain (3 probands), Molecular Genetics Unit, Casa Sollievo della Sofferenza Hospital, IRCSS, San Giovanni Rotondo, Italy (1 proband), Carlo Besta Neurological Institute, IRCSS, Milan, Italy (5 probands and 1 relative), University of Padova, Italy (2 probands), University Hospital, Ghent, Belgium (7 probands and 1 relative) and University of Leuven, Belgium (6 probands).

[C] **F**: **f**amilial; **PrS**: **pr**oven **s**poradic; **RS**: **r**eportedly **s**poradic; **NS**: **n**ot **s**pecified; **UN**: **un**known

[D] Exact age was used to calculate Height and Head Circumferences (HC), but provided as age-groups in Supporting Information Table S10: 0-24 months; 2-4 years; 5-8 years; 9-13 years; 14-18 years; 19-26 years; >26 years.

[E] **F**: **f**emale; **M**: **m**ale

[F] **W**: **W**hite; **His**: **His**panic; **AA**: **A**frican **A**merican; **As**: **As**ian; **NaA**: **Na**tive **A**merican; **A**: **A**frican; **O:** **O**ther

[G] An individual was classified as having Noonan-like phenotype if at least two of the following features were present: short stature (SS), low set ears (LSE), hypertelorism (HTL), midface hypoplasia (MH), webbed neck (WN), ptosis (PT), downslanting palpebral fissures (DPF), pectus abnormality (PA) or pulmonic stenosis (PS).

[H] Height percentiles for Hispanic and Asian individuals were provided in square brackets to indicate that they were excluded from the data analysis on frequency of short or normal stature due to the lack of ethnic-specific growth charts.

[I] Individuals with ADD/ADHD, but normal development were still classified as normal.

[J] RASopathy panel performed at UAB included 17 genes: *NF1, SPRED1, PTPN11, PPP1CB, BRAF, CBL, HRAS, KRAS, NRAS, MAP2K1, MAP2K2, RAF1, RIT1, RASA2, SHOC2, SOS1* and *SOS2*.

[K] Comprehensive alphabetical list of all genes mentioned in Supporting Information Table S10: *A2ML1* (MIM# 610627), *ACTB* (MIM# 102630), *ACTG1* (MIM# 102560), *APC* (MIM# 611731), *BRAF* (MIM# 164757), *CBL* (MIM# 165360), *CDC42* (MIM# 116952), *EPHB4* (MIM# 600011), *HRAS* (MIM# 190020), *KAT6B* (MIM# 605880), *KRAS* (MIM# 190070), *LZTR1* (MIM# 600574), *MAP2K1* (MIM# 176872), *MAP2K2* (MIM# 601263), *MUTYH* (MIM# 604933), *NF1* (MIM# 613113), *NRAS* (MIM# 164790), *POLD1* (MIM# 174761), *POLE* (MIM# 174762), *PPP1CB (MIM# 600590)*, *PTPN11* (MIM# 176876), *RAF1* (MIM# 164760), *RASA1* (MIM# 139150), *RASA2* (MIM# 601589), *RIT1* (MIM# 609591), *RRAS* (MIM# 165090), *SH3BP2* (MIM# 602104), *SHOC2* (MIM# 602775), *SOS1* (MIM# 182530), *SOS2* (MIM# 601247) and *SPRED1* (MIM# 609291).

Abbreviations: NS: **n**ot **s**pecified, i.e. no value provided on the phenotypic checklist; **UN**: **un**kown**; CALMs**: **c**afé-**a**u-**l**ait **m**acule**s**; **bil**: **bil**ateral; **gr**: **gr**oin; **ax**: **ax**illary; **sub**: **sub**mammary; **OPG**: **o**ptic **p**athway **g**lioma; **N, UN**: **n**o symptomatic OPG or symptomatic spinal neurofibroma, **un**known if any asymptomatic OPG or asymptomatic spinal neurofibromas are present; **N, MRI**: **n**o symptomatic and asymptomatic OPG or spinal neurofibroma detected by **MRI**; **Y, MRI**: **y**es, presence of OPG or spinal neurofibroma confirmed by **MRI**; **HTL**: **h**yper**t**e**l**orism; **WN**: short/**w**ebbed **n**eck; **MH**: **m**idface **h**ypoplasia; **LSE**: **l**ow **s**et **e**ars; **LPH**: **l**ow **p**osterior **h**airline; **SS**: **s**hort **s**tature; **PA**: **p**ectus **a**bnormality; **PT**: **pt**osis; **PS**: **p**ulmonic **s**tenosis; **DPF**: **d**ownslanting **p**alpebral **f**issures; **HC**: **h**ead **c**ircumference; **ADD**: **a**ttention **d**eficit **d**isorder; **ADHD**: **a**ttention **d**eficit **h**yperactivity **d**isorder; **ID**: **i**ntellectual **d**isability; **LD**: **l**earning **d**isability; **SD**: **s**peech **d**elay; **F**: **f**ather; **M**: **m**other; **S**: **s**ibling; **C**: **c**hild; **HS**: **h**alf-**s**ibling; **N**: **n**ephew; **aCGH**: **a**rray **c**omparative **g**enomic **h**ybridization; **FSIQ**: **F**ull **S**cale **I**ntelligence **Q**uotient; **MRI**: **m**agnetic **r**esonance **i**maging; **NGS**: **n**ext **g**eneration **s**equencing; **MPNST**: **m**alignant **p**eripheral **n**erve **s**heath **t**umor; **VUS**: **v**ariant of **u**ncertain **s**ignificance.

Supp. Table S11. Spectrum of clinical features in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276.

Pathogenic variant	**c.3826C>G (p.Arg1276Gly)**			**c.3826_3827delinsGA (p.Arg1276Glu)**			**c.3827G>A (p.Arg1276Gln)**			**c.3827G>C (p.Arg1276Pro)**			**c.3827G>T (p.Arg1276Leu)**			**All pathogenic variants**			**Total**
Pathogenic variant positive individuals (Proband:Relative)	16 (14:2)			2 (1:1)			93 (80:13)			3 (3:0)			5 (3:2)			119 (101:18)			119 (101:18)
Age group, years	**≤8**	**9-18**	**≥19**	**≤8**	**9-18**	**≥19**	**≤8**	**9-18**	**≥19**	**≤8**	**9-18**	**≥19**	**≤8**	**9-18**	**≥19**	**≤8**	**9-18**	**≥19**	**all ages**
Total	6	4	6	1	-	1	38	25	30	-	-	3	1	2	2	46	31	42	119
Proband:Relative	5:1	4:0	5:1	1:0	-	0:1	35:3	23:2	22:8	-	-	3:0	1:0	2:0	0:2	42:4	29:2	30:12	101:18
Male: Female	1:5	2:2	3:3	1:0	-	1:0	20:18	16:9	15:15	-	-	3:0	1:0	1:1	2:0	23:23	19:12	24:18	66:53
Fulfilling the NIH criteria if the family history is taken into account	2/6	4/4	6/6	1/1	-	1/1	26/36	18/24	28/30	-	-	3/3	0/1	2/2	2/2	29/44	24/30	40/42	93/116
Fulfilling the NIH criteria if solely taking the physical signs into account	2/6	4/4	6/6	0/1	-	1/1	22/36	16/24	28/30	-	-	3/3	0/1	2/2	2/2	24/44	22/30	40/42	86/116
>5 CALMs	4/6	4/4	6/6	1/1	-	1/1	37/38	22/25	28/30	-	-	3/3	1/1	2/2	2/2	43/46	28/31	40/42	111/119
Skinfold freckling	1/6	4/4	5/5	0/1	-	1/1	20/36	17/22	19/29	-	-	3/3	0/1	2/2	2/2	21/44	23/28	30/40	74/112
Lisch nodules	1/6	0/3	1/3	0/1	-	1/1	6/24	2/15	6/14	-	-	2/2	0/0	0/1	0/0	7/31	2/19	10/20	19/70
Skeletal abnormalities [A]	1/6	0/3	1/5	0/1	-	0/0	9/33	8/20	9/26	-	-	2/3	0/1	1/1	1/1	10/41	9/24	13/35	32/100
Plexiform neurofibromas	0/4	0/3	1/4	0/1	-	0/1	0/32	1/24	12/26	-	-	1/3	0/0	0/1	0/2	0/37	1/28	14/36	15/101
Cutaneous neurofibromas [B]	0/4	0/4	5/5	0/1	-	0/1	0/33	1/23	8/29	-	-	1/3	0/0	0/2	0/2	0/38	1/29	14/40	15/107
Subcutaneous neurofibromas [B]	0/2	0/4	0/2	0/1	-	1/1	0/32	1/23	18/29	-	-	2/3	0/0	0/1	0/2	0/35	1/28	21/37	22/100
Symptomatic spinal neurofibromas	0/3	1/3	1/4	0/1	-	0/1	0/30	0/22 [C]	15/27	-	-	1/2	0/0	0/2	0/2	0/34	1/27	17/36	18/97
Symptomatic OPG [D]	0/5	0/3	0/5	0/1	-	0/0	0/33	0/20	0/24	-	-	0/2	0/0	0/2	0/2	0/39	0/25	0/33	0/97
Asymptomatic OPG [E]	0/2	1/2	0/4	0/1	-	0/0	0/14	0/8	0/16	-	-	0/1	0/0	0/0	0/0	0/17	1/10	0/21	1/48
Other neoplasms [F]	0/5	0/3	3/5	0/1	-	0/1	0/30	2/18	2/26	-	-	1/3	0/0	0/0	0/2	0/36	2/21	6/37	8/94
Cognitive impairment and/or learning disabilities	4/6	3/4	2/5	0/1	-	0/0	12/36	14/22	6/23	-	-	1/3	1/1	2/2	1/2	17/44	19/28	10/33	46/105
Noonan-like phenotype [G]	1/6	1/4	0/4	0/1	-	0/0	10/36	7/23	3/25	-	-	0/3	0/1	0/2	0/1	11/44	8/29	3/33	22/106 [H]
Short stature [I]	0/5	1/4	0/3	0/1	-	0/0	6/25	4/17	3/21	-	-	0/2	0/0	0/1	0/1	6/31	5/22	3/27	14/80
Macrocephaly	3/5	1/4	0/2	0/1	-	0/0	12/30 [J]	5/16	3/14	-	-	0/1	0/1	0/1	0/1	15/37	6/21	3/18	24/76
Pulmonic stenosis	1/6	0/3	0/5	0/0	-	0/0	6/32	2/15	2/25	-	-	0/2	0/1	0/1	0/2	7/39	2/19	2/34	11/92
Cardiovascular abnormalities [K]	1/6	0/3	2/5	0/0	-	0/0	9/32	4/15	5/25	-	-	0/2	0/1	0/1	1/2	10/39	4/19	8/34	22/92

[A] All bone abnormalities included, that is, scoliosis (n=17), pectus abnormality (n=13), sphenoid wing dysplasia (n=2), leg or limb length discrepancy (n=2), marfanoid habitus (n=1), kyphosis (n=1), cherubism (n=1) and osteoporosis (n=1). [B] At least two cutaneous/subcutaneous neurofibromas were required to be considered as "positive for the criterion of neurofibromas". [C] Excluding two individuals (UAB-R0843 and UAB-R7373) with *possible* symptomatic spinal neurofibromas, but no further clinical details available. [D] The absence of symptomatic OPGs was determined by ophthalmological examination and/or by MRI. [E] Including only individuals without signs of symptomatic OPGs who underwent MRI examination. [F] All neoplasms, excluding OPGs and neurofibromas, included, that is, astrocytoma (n=2), other glioma than OPG (n=1), pheochromocytoma (n=1), MPNST (n=1), colon cancer (n=1), meningioma (n=1), lipoma (n=1) and supratentorial anaplastic ependymoma (n=1). [G] An individual was classified as having Noonan-like phenotype when at least two of the following features were present: short stature, low set ears, hypertelorism, downslanting palpebral fissures, midface hypoplasia, ptosis, webbed neck, pectus abnormality and/or pulmonic stenosis. [H] Excluding 14 individuals with *possible* Noonan-like features, but further clinical details were not available. [I] As no specific growth curves are available for the Hispanic and Asian populations, Hispanic and Asian individuals were excluded as having short or normal stature. [J] Including a single individual (UAB-R9514) with head circumference PC97 classified as a macrocephaly. [K] All cardiovascular abnormalities included, that is, pulmonic stenosis (n=11), hypertension (n=5), aortic stenosis (n=4), aortic septal defect (n=2), myocardial infarction (n=1), Moya moya disease (n=1), left middle aortic stenosis (=1), hypertrophic cardiomyopathy (n=1), ventricular septal defect (n=1), tricuspid artresia (n=1), hypoplastic right heart syndrome (n=1), renal artery stenosis (n=1), left ventricular hypertrophy (n=1), carotid artery stenosis (n=1), coronary disease (n=1), atrial fibrillation (n=1) and stroke (n=1).

Supp. Table S12. Clinical details for 94 individuals with one of four different amino substitutions at *NF1* p.Lys1423 from 87 different families.

Supp. Table S12 is included as a separate Excel file.

[A] For individuals with one asterisk (*) the standardized phenotypic checklist forms were not re-verified/updated by the referring physicians, the data are based on the originally submitted forms; individuals with two asterisks (**) had incomplete phenotypic checklist forms.

[B] **UAB**: **U**niversity of **A**labama at **B**irmingham (64 probands and 2 relatives); **EUR**: individuals referred by the **Eur**opean collaborators (23 probands and 5 relatives), including Medical University of Innsbruck, Austria (3 probands and 1 relative), Hospital Universitario Ramón y Cajal, IRYCIS and CIBERER, Madrid, Spain (2 probands), Molecular Genetics Unit, Casa Sollievo della Sofferenza Hospital, IRCSS, San Giovanni Rotondo, Italy (7 probands and 3 relatives), Carlo Besta Neurological Institute, IRCSS, Milan, Italy (5 probands), University of Padova, Italy (3 probands), University Hospital, Ghent, Belgium (2 probands and 1 relative) and University of Leuven, Belgium (1 proband).

[C] **F**: **f**amilial; **PrS**: **pr**oven **s**poradic; **RS**: **r**eportedly **s**poradic; **NS**: **n**ot **s**pecified; **UN**: **un**known

[D] Exact age was used to calculate Height and Head Circumferences (HC), but provided as age-groups in Supporting Information Table S12: 0-24 months; 2-4 years; 5-8 years; 9-13 years; 14-18 years; 19-26 years; >26 years.

[E] **F**: **f**emale; **M**: **m**ale

[F] **W**: **W**hite; **His**: **His**panic; **AA**: **A**frican **A**merican; **As**: **As**ian; **NaA**: **Na**tive **A**merican; **O:** **O**ther

[G] An individual was classified as having „Noonan-like" phenotype if at least two of the following features were present: short stature (SS), low set ears (LSE), hypertelorism (HTL), midface hypoplasia (MH), webbed neck (WN), ptosis (PT), downslanting palpebral fissures (DPF), pectus abnormality (PA) or pulmonic stenosis (PS).

[H] Height percentiles for Hispanic and Asian individuals were provided in square brackets to indicate that they were excluded from the data analysis on frequency of short or normal stature due to the lack of ethnic-specific growth charts.

[I] Individuals with ADD/ADHD, but normal development were still classified as normal.

[J] RASopathy panel performed at UAB included 17 genes: *NF1, SPRED1, PTPN11, PPP1CB, BRAF, CBL, HRAS, KRAS, NRAS, MAP2K1, MAP2K2, RAF1, RIT1, RASA2, SHOC2, SOS1* and *SOS2*.

[K] Comprehensive alphabetical list of all genes mentioned in Supporting Information Table S12: *A2ML1* (MIM# 610627), *BRAF* (MIM# 164757), *CBL* (MIM# 165360), *CDC42* (MIM# 116952), *HRAS* (MIM# 190020), *IDH1* (MIM# 147700), *KRAS* (MIM# 190070), *LZTR1* (MIM# 600574), *MAP2K1* (MIM# 176872), *MAP2K2* (MIM# 601263), *NF1* (MIM# 613113), *NRAS* (MIM# 164790), *PPP1CB* (MIM# 600590), *PTPN11* (MIM# 176876), *RAF1* (MIM# 164760), *RASA1* (MIM# 139150), *RASA2* (MIM# 601589), *RIT1* (MIM# 609591), *RRAS* (MIM# 165090), *SHOC2* (MIM# 602775), *SOS1* (MIM# 182530), *SOS2* (MIM# 601247) and *SPRED1* (MIM# 609291).

[L] UAB-R1753, carrying c.4268A>T (p.Lys1423Met), was excluded from the genotype-phenotype study as the interpretation of this specific variant is "*likely pathogenic"* according to the ACMG recommendations (see details in Supporting Information Table S5).

Abbreviations: NS: **n**ot **s**pecified, i.e. no value provided on the phenotypic checklist; **UN**: **un**kown**; CALMs**: **c**afé-**a**u-**l**ait **m**acule**s**; **bil**: **bil**ateral; **gr**: **gr**oin; **ax**: **ax**illary; **sub**: **sub**mammary; **OPG**: **o**ptic **p**athway **g**lioma; **N, UN**: **n**o symptomatic OPG or symptomatic spinal neurofibroma, **un**known if any asymptomatic OPG or asymptomatic spinal neurofibromas are present; **N, MRI**: **n**o symptomatic and asymptomatic OPG or spinal neurofibroma detected by **MRI**; **Y, MRI**: **y**es, presence of OPG or spinal neurofibroma confirmed by **MRI**; **HTL**: **h**yper**t**e**l**orism; **WN**: short/**w**ebbed **n**eck; **MH**: **m**idface **h**ypoplasia; **LSE**: **l**ow **s**et **e**ars; **LPH**: **l**ow **p**osterior **h**airline; **SS**: **s**hort **s**tature; **PA**: **p**ectus **a**bnormality; **PT**: **pt**osis; **PS**: **p**ulmonic **s**tenosis; **DPF**: **d**ownslanting **p**alpebral **f**issures; **HC**: **h**ead **c**ircumference; **ADD**: **a**ttention **d**eficit **d**isorder; **ADHD**: **a**ttention **d**eficit **h**yperactivity **d**isorder; **LD**: **l**earning **d**isability; **SD**: **s**peech **d**elay; **M**: **m**other; **S**: **s**ibling; **C**: **c**hild; **FSIQ**: **F**ull **S**cale **I**ntelligence **Q**uotient; **MRI**: **m**agnetic **r**esonance **i**maging; **NGS**: **n**ext **g**eneration **s**equencing; **PNST**: **p**eripheral **n**erve **s**heath **t**umor.

Supp. Table S13. Spectrum of clinical features in individuals heterozygous for the *NF1* missense pathogenic variants affecting p.Lys1423.

Pathogenic variant	**c.4267A>C (p.Lys1423Gln)**			**c.4267A>G (p.Lys1423Glu)**			**c.4268A>C (p.Lys1423Thr)**			**All pathogenic variants**			**Total**
Pathogenic variant positive individuals (Proband:Relative)	7 (7:0)			84 (77:7)			2 (2:0)			93 (86:7)			93 (86:7)
Age group, years	**≤8**	**9-18**	**≥19**	**≤8**	**9-18**	**≥19**	**≤8**	**9-18**	**≥19**	**≤8**	**9-18**	**≥19**	**all ages**
Total	3	2	2	32	21	31	1	1	-	36	24	33	93
Proband:Relative	3:0	2:0	2:0	32:0	20:1	25:6	1:0	1:0	-	36:0	23:1	27:6	86:7
Male: Female	1:2	1:1	0:2	17:15	13:8	15:16	1:0	1:0	-	19:17	15:9	15:18	49:44
Fulfilling the NIH criteria if the family history is taken into account	2/2	2/2	2/2	21/29	21/21	31/31	1/1	1/1	-	24/32	24/24	33/33	81/89
Fulfilling the NIH criteria if solely taking the physical signs into account	2/2	2/2	2/2	18/29	20/21	31/31	1/1	0/1	-	21/32	22/24	33/33	76/89
>5 CALMs	2/2	2/2	2/2	27/31	21/21	30/31	1/1	1/1	-	30/34	24/24	32/33	86/91
Skinfold freckling	2/2	2/2	2/2	19/29	17/21	22/28	1/1	0/0	-	22/32	19/23	24/30	65/85
Lisch nodules	0/1	0/1	0/1	6/21	6/13	19/22	0/0	0/0	-	6/22	6/14	19/23	31/59
Skeletal abnormalities [A]	0/3	0/2	2/2	7/28	12/21	13/25	0/1	0/1	-	7/32	12/24	15/27	34/83
Plexiform neurofibromas	0/3	0/2	1/1	4/27	6/19	8/25	0/1	0/1	-	4/31	6/22	9/26	19/79
Cutaneous neurofibromas [B]	0/2	0/2	1/1	3/27	2/19	22/27	0/1	0/1	-	3/30	2/22	23/28	28/80
Subcutaneous neurofibromas [B]	0/1	0/2	0/1	3/26	1/18	13/22	0/0	0/1	-	3/27	1/21	13/23	17/71
Symptomatic spinal neurofibromas	0/2	0/2	0/1	2/23	0/14	1/21	0/1	0/1	-	2/26	0/17	1/22	3/65
Symptomatic OPG [C]	0/3	0/2	0/1	0/21	0/20	1/25	0/1	0/1	-	0/25	0/23	1/26	1/74
Asymptomatic OPG [D]	0/2	0/1	0/1	1/8	4/13	1/14	0/0	0/1	-	1/10	4/15	1/15	6/40
Other neoplasms [E]	0/3	0/2	1/1	1/24	1/18	8/27	0/1	0/1	-	1/28	1/21	9/28	11/77
Cognitive impairment and/or learning disabilities	1/3	1/2	1/2	14/31	13/19	6/28	0/1	0/1	-	15/35	14/22	7/30	36/87
Noonan-like phenotype [F]	0/3	1/2	0/1	10/30	8/19	4/27	1/1	0/0	-	11/34	9/21	4/28	24/83 [G]
Short stature [H]	0/0	0/1	1/1	5/15	5/15	10/19	0/0	0/0	-	5/15	5/16	11/20	21/51
Macrocephaly	0/1	0/0	0/1	4/18	5/14	6/16	0/1	0/0	-	4/20	5/14	6/17	15/51
Pulmonic stenosis	1/3	0/2	0/1	4/24	5/20	1/25	0/0	0/1	-	5/27	5/23	1/26	11/76
Cardiovascular abnormalities [I]	1/3	0/2	0/1	5/24	8/20	5/25	0/0	0/1	-	6/27	8/23	5/26	19/76

[A] All bone abnormalities included, that is, scoliosis (n=17), pectus abnormality (n=10), pes planus (n=3), kyphosis (n=2), bone cysts (n=2), lordosis (n=1), sphenoid wing dysplasia (n=1), long bone dysplasia (n=1), cubitus valgus (n=1), osteoporosis (n=1), dysplastic vertebrae (n=1) and pseudoarthrosis (n=1). [B] At least two cutaneous/subcutaneous neurofibromas were required to be considered as "positive for the criterion of neurofibromas". [C] The absence of symptomatic OPGs was determined by ophthalmological examination and/or by MRI. [D] Including only individuals without signs of symptomatic OPGs who underwent MRI examination. [E] All neoplasms, excluding OPGs and neurofibromas, included, that is, non-ossifying fibromas (n=3), giant cell tumor (n=1), rhabdomyomysarcoma (n=1), peripheral nerve sheath tumor (n=1), colon adenocarcinoma (n=1), meningioma (n=1), cerebellar glioblastoma (n=1), pilocytic astrocytoma (n=1), hypothalamic glioma (n=2), low grade glioma (n=2) and brainstem glioma (n=1). [F] An individual was classified as having Noonan-like phenotype when at least two of the following features were present: short stature, low set ears, hypertelorism, downslanting palpebral fissures, midface hypoplasia, ptosis, webbed neck, pectus abnormality and/or pulmonic stenosis. [G] Excluding three individuals with *possible* Noonan-like features, but further clinical details were not available. [H] As no specific growth curves are available for the Hispanic and Asian populations, Hispanic and Asian individuals were excluded as having short or normal stature. [I] All cardiovascular abnormalities included, that is, pulmonic stenosis (n=11), hypertension (n=2), ventricular septal defect (n=2), Moya moya disease, mitral valve anomaly, aortic stenosis, Wolff-Parkinson-White syndrome, dilated ventricles, bicuspid aortic valve, aortic reguduralrgitation, aortic root dilatation and parachute deformity of mitral valve (all observed in a single individual.

Supp. Table S14. Spinal neurofibromas in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276.

ID [A]	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Neurofibromas	Others	Comments
SYMPTOMATIC SPINAL NEUROFIBROMAS								
UAB-R0193	c.3826C>G (p.Arg1276Gly)	F	14-18	>5	+	-	*possible* Noonan-like phenotype	tumors on all spinal nerve roots
EUR-R10	c.3826C>G (p.Arg1276Gly)	RS	19-26	>5	+	internal plexiform and 6-99 cutaneous neurofibromas	Lisch nodules, MPNST	bilateral in cervical C1-8, thoracic T1-12, lumbar L1-5 and sacral S1-6 nerves
UAB-R0306	c.3827G>A (p.Arg1276Gln)	PrS	19-26	>5	+	multiple internal plexiform and subcutaneous neurofibromas	Lisch nodules, ADD, ADHD, LD, cherubism, severe osteoporosis, SS	unilateral tumors in T10-S5 regions
UAB-R492	c.3827G>A (p.Arg1276Gln)	NS	19-26	+	+	plexiform neurofibromas and multiple cutaneous/subcutaneous neurofibromas	cystic lesion	spine MRI: diffuse extensive involvement of neurofibromas along the course of the peripheral nerves
EUR-R22	c.3827G>A (p.Arg1276Gln)	F	19-26	>5	+	plexiform neurofibroma, ~30 cutaneous and 6-99 subcutaneous neurofibromas	Lisch nodules, pectus excavatum	bilateral tumors on all spinal nerve roots
EUR-R28	c.3827G>A (p.Arg1276Gln)	F	19-26	>5	+	internal plexiform and 2-6 subcutaneous neurofibromas, *histopathologically confirmed*	Lisch nodules, cysts in brain, nevi/lentigines	cervical C1-2 and C7, thoracic T1 and T11-12, lumbar L1-5 and sacral S1-5 nerves, *histopathologically confirmed*
UAB-R7942	c.3827G>A (p.Arg1276Gln)	F	>26	>5	+	internal plexiform and >500 subcutaneous neurofibromas	SS	bilateral tumors on all spinal nerve roots
UAB-R0215-F	c.3827G>A (p.Arg1276Gln)	F	>26	>5	+	-	headaches with dizziness, joint stiffness, morbid obesity	multiple tumors; individual had pain at multiple sites (lower back, right knee and chest)
UAB-R1256-M	c.3827G>A (p.Arg1276Gln)	F	>26	>5	+	2-6 cutaneous, 2-6 subcutaneous and 2-6 intradermal neurofibromas, *histopathologically confirmed*	scoliosis, aortic septal defect, LD, speech therapy	bilateral tumors in thoracic nerves
UAB-R01611FN.104	c.3827G>A (p.Arg1276Gln)	UN	>26	-	-	internal plexiform and 2-6 subcutaneous neurofibromas	-	cervical and lumbar nerves, *histopathologically confirmed*
UAB-R743	c.3827G>A (p.Arg1276Gln)	NS	>26	<6	-	multiple internal plexiform and subcutaneous neurofibromas	LD	tumors on all spinal nerve roots, *histopathologically confirmed*; individual had pain and weakness of arms and legs, increasing over the years; case reported by Korf et al. (2005)
UAB-R743-N	c.3827G>A (p.Arg1276Gln)	F	>26	>5	+	multiple internal plexiform and subcutaneous neurofibromas, *histopathologically confirmed*	-	individual had pain and weakness of the left leg; a single lesion was surgically removed and *histopathologically confirmed*; case reported by Korf et al. (2005)
EUR-R21-F	c.3827G>A (p.Arg1276Gln)	RS	>26	>5	+	6-99 subcutaneous neurofibromas, *histopathologically confirmed*	hypertension, colonic polyps, colon cancer, macrocephaly	bilateral, tumors in cervical C1-8, thoracic T1-12, lumbar L1-5 and sacral S1-5 nerves, *histopathologically confirmed*

SYMPTOMATIC SPINAL NEUROFIBROMAS								
EUR-R22-F	c.3827G>A (p.Arg1276Gln)	F	>26 †	>5	+	~20 cutaneous and 6-99 subcutaneous neurofibromas	-	diffuse symmetrical tumors along cervical and thoracic nerves; individual died at age of 69 due to the subarachnoid hemorrhage
EUR-R26	c.3827G>A (p.Arg1276Gln)	F	>26	>5	-	-	scoliosis, osteopenia, hypertension, pulmonic stenosis, *possible* Noonan-like phenotype, SS	tumors in cervical C4, thoracic T2, lumbar L2 and sacral S1 nerves
EUR-R27	c.3827G>A (p.Arg1276Gln)	PrS	>26	>5	-	2-6 subcutaneous neurofibromas, *histopathologically confirmed*	-	tumors in cervical C2-3 and C7, thoracic T1, lumbar and sacral nerves, *histopathologically confirmed*
EUR-R29	c.3827G>A (p.Arg1276Gln)	RS	>26	>5	NS	NS	individual was operated at age of ~30 year due to posterior mediastinum neurofibromas	tumors in lumbar and sacral nerves
EUR-R32	c.3827G>C (p.Arg1276Pro)	RS	>26	>5	+	100-500 cutaneous, 6-99 subcutaneous and 2-6 intradermal neurofibromas	Lisch nodules, scoliosis, pheochromocytoma	diffuse symmetrical tumors along all spinal roots, *histopathologically confirmed*
ASYMPTOMATIC SPINAL NEUROFIBROMAS								
UAB-R0522	c.3827G>A (p.Arg1276Gln)	F	19-26	>5	+	2-6 cutaneous neurofibromas	-	tumors on all spinal nerve roots
EUR-R17	c.3827G>A (p.Arg1276Gln)	RS	>26	>5	-	2-6 intradermal neurofibromas	-	tumors in cervical C3-7, thoracic T1-2 and T10-12 and lumbar L1-3 nerves
EUR-R23	c.3827G>A (p.Arg1276Gln)	RS	>26	>5	-	internal plexiform and 2-6 subcutaneous neurofibromas, *histopathologically confirmed*	scoliosis, hypertension	bilateral tumors in sacral nerves
EUR-R24	c.3827G>A (p.Arg1276Gln)	RS	>26	>5	-	plexiform, 3 cutaneous and 6-99 subcutaneous neurofibromas, *histopathologically confirmed*	Lisch nodules, pulmonic stenosis	diffuse symmetrical tumors on all spinal nerve roots

[A] IDs of individuals presenting the so-called spinal form of NF1 were bolded.

Abbreviations: F - **f**amilial; **PrS** - **pr**oven **s**poradic; **RS** - **r**eportedly **s**poradic; **NS** - **n**ot **s**pecified; **UN** - **un**known; **CALMs** - **c**afé-**a**u-**l**ait **m**acule**s**; **ADD** - **a**ttention **d**eficit **d**isorder; **ADHD** - **a**ttention **d**eficit **h**yperactivity **d**isorder; **LD** - **l**earning **d**isabilities; **SS** - **s**hort **s**tature; **MPNST** - **m**alignant **p**eripheral **n**erve **s**heath **t**umor; **MRI** - **m**agnetic **r**esonance **i**maging.

Supp. Table S15. Spinal neurofibromas in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Lys1423.

ID	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Neurofibromas	Others	Comments
SYMPTOMATIC SPINAL NEUROFIBROMAS								
UAB-R5344	c.4267A>G (p.Lys1423Glu)	PrS	<2	-	-	internal and externally visible plexiform neurofibromas, *histopathologically confirmed*	scoliosis, SS	bilateral tumors in thoracic (T1-8) and lumbar (L1-4) nerves as well as cauda equina, *histopathologically confirmed*
UAB-R2741	c.4267A>G (p.Lys1423Glu)	PrS	5-8	>5	+	multiple small subcutaneous neurofibromas	Noonan-like phenotype, SD, hypotonia, macrocephaly, headaches, neck pain	tumor in the left thoracic region with no evidence of invasion of spinal canal; neurologic exam was notable for normal cranial nerves but muscular hypotonia
EUR-R53	c.4267A>G (p.Lys1423Glu)	RS	>26 †	<6	+	100-500 cutaneous, 100-500 subcutaneous and 100-500 intradermal neurofibromas	Lisch nodules, delayed for age, SS, individual died at age of 47 due to progression of cerebellar glioblastoma (*histopathologically confirmed*)	diffuse symmetrical tumors on all spinal nerve roots
ASYMPTOMATIC SPINAL NEUROFIBROMAS								
EUR-R41	c.4267A>G (p.Lys1423Glu)	RS	5-8	>5	+	externally visible plexiform neurofibromas, 6-99 cutaneous and 2-6 intradermal neurofibromas	scoliosis, pectus excavatum, gross and fine motor delays, delayed for age, hypotonic, SD	bilateral tumors on all spinal levels (cervical C2-3, thoracic T10-12, lumbar L2-5 and sacral S1-4 nerves)
UAB-R1185	c.4267A>G (p.Lys1423Glu)	PrS	19-26	>5	-	6-99 cutaneous neurofibromas	*possible* Noonan-like phenotype, pilocytic astrocytoma in thalamus (grade I) and bilateral non-ossifying fibromas in knees	multiple tumors at each spinal level, innumerable
EUR-R50	c.4267A>G (p.Lys1423Glu)	UN	19-26	>5	+	-	Lisch nodules, asymptomatic OPG	tumor in cervical nerve C7
UAB-R97511FN.204	c.4267A>G (p.Lys1423Glu)	NS	>26	>5	+	6-99 cutaneous neurofibromas	Noonan-like phenotype, macrocephaly, brainstem glioma	bilateral tumors in lumbar nerves

Abbreviations: F - **f**amilial; **PrS** - **pr**oven **s**poradic; **RS** - **r**eportedly **s**poradic; **NS** - **n**ot **s**pecified; **UN** - **un**known; **CALMs** - **c**afé-**a**u-**l**ait **m**acule**s**; **SS** - **s**hort **s**tature; **SD** - **s**peech **d**isability; **OPG** - **o**ptic **p**athway **g**lioma.

Supp. Table S16. Plexiform neurofibromas in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Lys1423.

ID	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Others	Comments
UAB-R805	c.4267A>G (p.Lys1423Glu)	NS	<2	>5	+	-	**PLEXIFORM NEUROFIBROMAS** orbit, face and head/neck
UAB-R5344	c.4267A>G (p.Lys1423Glu)	PrS	<2	-	-	symptomatic spinal neurofibromas, scoliosis, SS	**PLEXIFORM NEUROFIBROMA** left back (internal and externally visible), *histopathologically confirmed*
EUR-R38	c.4267A>G (p.Lys1423Glu)	RS	2-4	>5	+	Lisch nodules, sphenoid wing dysplasia, Noonan-like phenotype, ADHD	**PLEXIFORM NEUROFIBROMA** head (externally visible without hyperpigmentation)
EUR-R41	c.4267A>G (p.Lys1423Glu)	RS	5-8	>5	+	cutaneous, intradermal and spinal neurofibromas, scoliosis, pectus excavatum, gross and fine motor delays, hypotonic, SD	**PLEXIFORM NEUROFIBROMAS** head and neck (externally visible without hyperpigmentation and internal)
UAB-R211	c.4267A>G (p.Lys1423Glu)	NS	9-13	>5	+	pseudoarthrosis	**PLEXIFORM NEUROFIBROMA** trunk
UAB-R746	c.4267A>G (p.Lys1423Glu)	RS	9-13	>5	-	cutaneous and subcutaneous neurofibromas, mild pectus excavatum, Noonan-like phenotype, abnormal development, ADHD	**PLEXIFORM NEUROFIBROMAS** leg and trunk (dorsal)
UAB-R7403	c.4267A>G (p.Lys1423Glu)	RS	14-18	>5	+	Noonan-like phenotype, pulmonic stenosis, ADD	**PLEXIFORM NEUROFIBROMA** head (externally visible with hyperpigmentation)
UAB-R2816	c.4267A>G (p.Lys1423Glu)	RS	14-18	>5	+	Lisch nodules, macrocephaly	**PLEXIFORM NEUROFIBROMA** right arm (with hyperpigmentation)
UAB-R6707	c.4267A>G (p.Lys1423Glu)	F	14-18	>5	+	scoliosis, SS, macrocephaly, Chiari malformation	**PLEXIFORM NEUROFIBROMA** right leg (externally visible with hyperpigmentation)
UAB-R2551	c.4267A>G (p.Lys1423Glu)	RS	14-18	>5	+	Noonan-like phenotype, dysplastic vertebrae, lordosis and kyphosis, pulmonic stenosis, leg pain, seizures, a mild tremor	**PLEXIFORM NEUROFIBROMAS** occiput and neck (internal)
EUR-R51	c.4267A>G (p.Lys1423Glu)	RS	19-26	>5	+	Lisch nodules, bone cysts, delayed for age with severe behavior disorder	**PLEXIFORM NEUROFIBROMA** trunk (externally visible)
UAB-R53601FN.103	c.4267A>G (p.Lys1423Glu)	RS	19-26	>5	+	Lisch nodules, cutaneous neurofibromas	**PLEXIFORM NEUROFIBROMA** left arm
EUR-R35	c.4267A>C (p.Lys1423Gln)	RS	>26	>5	+	cutaneous neurofibromas, mild scoliosis, SS, low grade glioma, LD, joint laxity	**PLEXIFORM NEUROFIBROMA** (externally visible with hyperpigmentation)
UAB-R6071	c.4267A>G (p.Lys1423Glu)	RS	>26	>5	+	Lisch nodules, cutaneous and intradermal neurofibromas, LD	**PLEXIFORM NEUROFIBROMAS** head
UAB-R5232	c.4267A>G (p.Lys1423Glu)	UN	>26	>5	UN	cutaneous, intradermal and subcutaneous neurofibromas, scoliosis	**PLEXIFORM NEUROFIBROMAS** right arm (externally visible)
UAB-R3903	c.4267A>G (p.Lys1423Glu)	F	>26	>5	+	Lisch nodules, cutaneous, intradermal and subcutaneous neurofibromas, SS, LD	**PLEXIFORM NEUROFIBROMAS** left arm and right arm (externally visible)
EUR-R47	c.4267A>G (p.Lys1423Glu)	F	>26	>5	NS	cutaneous and subcutaneous neurofibromas, scoliosis, macrocephaly	**PLEXIFORM NEUROFIBROMA** left arm (externally visible)
EUR-R48-S1	c.4267A>G (p.Lys1423Glu)	F	>26	>5	+	scoliosis, SS	**PLEXIFORM NEUROFIBROMA** head (externally visible)
EUR-R48-M	c.4267A>G (p.Lys1423Glu)	F	>26	>5	+	cutaneous and subcutaneous neurofibromas, scoliosis, SS	**PLEXIFORM NEUROFIBROMA** parietal region (externally visible)

Abbreviations: F - **f**amilial; **PrS** - **pr**oven **s**poradic; **RS** - **r**eportedly **s**poradic; **NS** - **n**ot **s**pecified; **UN** - **un**known; **CALMs** - **c**afé-**a**u-**l**ait **m**acule**s**; **SS** - **s**hort **s**tature; **ADD** - **a**ttention **d**eficit **d**isorder; **ADHD** - **a**ttention **d**eficit **h**yperactivity **d**isorder; **SD** - **s**peech **d**elay; **LD** - **l**earning **d**isabilities.

Supp. Table S17. Plexiform neurofibromas in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276.

ID	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Others	Comments
UAB-R7373	c.3827G>A (p.Arg1276Gln)	RS	9-13	>5	+	Lisch nodules, ADD, ADHD, LD	**PLEXIFORM NEUROFIBROMA** T9-T10 right inferior, confirmed to be a lumbosacral plexiform neurofibroma with pain
EUR-R10	c.3826C>G (p.Arg1276Gly)	RS	19-26	>5	+	Lisch nodules, cutaneous and symptomatic spinal neurofibromas, MPNST	**PLEXIFORM NEUROFIBROMAS** left arm and right leg (internal)
UAB-R4762	c.3827G>A (p.Arg1276Gln)	RS	19-26	>5	-	pectus excavatum, Noonan-like phenotype, macrocephaly	**PLEXIFORM NEUROFIBROMA** ureter (internal), *histopathologically confirmed*
UAB-R0306	c.3827G>A (p.Arg1276Gln)	PrS	19-26	>5	+	Lisch nodules, multiple subcutaneous and symptomatic spinal neurofibromas, cherubism, severe osteoporosis, left hydronephrosis, mild myopia, spina bifida occulta at S1, a history of migraines and occasional right hand numbness, SS	**PLEXIFORM NEUROFIBROMAS** multiple tumors with intra-abdominal, paraspinal, intraspinal, abdominal wall and chest wall locations (internal)
UAB-R492	c.3827G>A (p.Arg1276Gln)	NS	19-26	+	+	multiple cutaneous, subcutaneous and symptomatic spinal neurofibromas, cystic lesion	**PLEXIFORM NEUROFIBROMAS** head/neck, trunk, arm and leg
EUR-R22	c.3827G>A (p.Arg1276Gln)	F	19-26	>5	+	Lisch nodules, cutaneous, subcutaneous and symptomatic spinal neurofibromas, pectus excavatum	**PLEXIFORM NEUROFIBROMA** right foot (externally visible)
EUR-R28	c.3827G>A (p.Arg1276Gln)	F	19-26	>5	+	subcutaneous and symptomatic spinal neurofibromas, *histopathologically confirmed*, cysts in brain, nevi/lentigines	**PLEXIFORM NEUROFIBROMA** pelvis (internal), *histopathologically confirmed*
EUR-R31	c.3827G>C (p.Arg1276Pro)	RS	19-26	>5	+	intradermal neurofibromas, pectus excavatum, broad chest/telethelia, LD, nevi/lentigines	**PLEXIFORM NEUROFIBROMA** trunk (externally visible without hyperpigmentation)
UAB-R7942	c.3827G>A (p.Arg1276Gln)	F	>26	>5	+	subcutaneous and symptomatic spinal neurofibromas, juvenile xanthogranuloma	**PLEXIFORM NEUROFIBROMA** pelvis (internal)
UAB-R01611FN.104	c.3827G>A (p.Arg1276Gln)	UN	>26	-	-	subcutaneous and symptomatic spinal neurofibromas	**PLEXIFORM NEUROFIBROMA** (internal)
UAB-R743	c.3827G>A (p.Arg1276Gln)	NS	>26	<6	-	symptomatic spinal neurofibromas	**PLEXIFORM NEUROFIBROMAS** trunk, pelvis and sciatic nerve (internal)
UAB-R743-N	c.3827G>A (p.Arg1276Gln)	F	>26	>5	+	subcutaneous and symptomatic spinal neurofibromas	**PLEXIFORM NEUROFIBROMAS** (internal)
EUR-R13	c.3827G>A (p.Arg1276Gln)	F	>26	>5	-	-	**PLEXIFORM NEUROFIBROMA** the nipple-areola complex (externally visible), *histopathologically confirmed*
EUR-R23	c.3827G>A (p.Arg1276Gln)	RS	>26	>5	-	subcutaneous and asymptomatic spinal neurofibromas, scoliosis, hypertension	**PLEXIFORM NEUROFIBROMAS** pelvis and right leg (internal), *histopathologically confirmed*
EUR-R24	c.3827G>A (p.Arg1276Gln)	RS	>26	>5	-	Lisch nodules, cutaneous, subcutaneous and asymptomatic spinal neurofibromas, pulmonic stenosis	**PLEXIFORM NEUROFIBROMA** left leg (externally visible)

Abbreviations: F - **f**amilial; **PrS** - **pr**oven **s**poradic; **RS** - **r**eportedly **s**poradic; **NS** - **n**ot **s**pecified; **UN** - **un**known; **ADD** - **a**ttention **d**eficit **d**isorder; **ADHD** - **a**ttention **d**eficit **h**yperactivity **d**isorder; **LD** - **l**earning **d**isabilities; **MPNST** - **m**alignant **p**eripheral **n**erve **s**heath **t**umor; **SS** - **s**hort **s**tature.

Supp. Table S18. Comparison of clinical features of the cohort of individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276 and p.Lys1423 with the cohort of the individuals carrying one out of the 18 most frequent recurrent pathogenic nonsense variants observed in the University of Alabama at Birmingham (UAB) database.

	N (%)			*P* value (2-tailed Fisher's exact test)		
	p.Arg1276	**p.Lys1423**	**Nonsense variants** [A]	**p.Arg1276 versus p.Lys1423**	**p.Arg1276 versus nonsense variants**	**p.Lys1423 versus nonsense variants**
>5 CALMs	111/119 (93.3)	86/91 (94.5)	855/908 (94.2)			
Skinfold freckling	74/112 (66.1)	65/85 (76.5)	589/834 (70.6)			
Lisch nodules	19/70 (24.1)	31/59 (52.5)	140/474 (29.5)	0.0038* ↘		0.0006** ↗
Major external plexiform neurofibromas [B]	5/64 (7.8)	14/48 (29.2)	44/329 (13.4)	0.0044* ↘		0.0089* ↗
Cutaneous neurofibromas [C]	14/40 (35)	23/28 (82.1)	202/237 (85.2)	0.0002** ↘	<0.0001** ↘	
Subcutaneous neurofibromas [C]	21/37 (56.8)	13/23 (56.5)	76/138 (55.1)			
Symptomatic spinal neurofibromas	18/97 (18.6)	3/65 (4.6)	17/728 (2.3)	0.0091* ↗	<0.0001** ↗	
Symptomatic OPGs	0/97 (0)	1/74 (1.4)	45/802 (5.6)		0.0109* ↘	
Asymptomatic OPGs	1/48 (2.1)	6/40 (15)	41/191 (21.5)		0.0006** ↘	
Other malignant neoplasms [D]	4/94 (4.3)	7/77 (9.1)	33/742 (4.5)			
Skeletal abnormalities	32/100 (32)	34/83 (41)	151/811 (18.6)		0.0033* ↗	<0.0001** ↗
Scoliosis [C]	8/35 (22.9)	10/27 (38.5)	29/232 (12.5)			0.0024* ↗
Cognitive impairment and/or learning disabilities	46/105 (43.8)	36/87 (41.4)	215/790 (27.2)		0.0008** ↗	0.0083* ↗
Noonan-like phenotype [E]	22/106 (20.8)	24/83 (28.9)	14/773 (1.8)		<0.0001** ↗	<0.0001** ↗
Short stature [F]	14/80 (17.5)	21/51 (41.2)	59/362 (16.3)	0.0043* ↘		0.0001** ↗
Macrocephaly	24/76 (31.6)	15/51 (29.4)	113/405 (27.9)			
Pulmonic stenosis	11/92 (12)	11/76 (14.5)	13/701 (1.9)		<0.0001** ↗	<0.0001** ↗
Cardiovascular abnormalities	22/92 (23.9)	19/76 (25)	52/701 (7.4)		<0.0001** ↗	<0.0001** ↗

Statystically significant *P* values with false discovery rate (FDR) of 0.05 (indicated by *) and 0.01 (indicated by **) after correction for multiple testing using Benjamini-Hochberg procedure. After applying the Benjamini-Hochberg correction *P*≤0.0091 and *P*≤0.0008 remained statistically significant at FDR 0.05 and 0.01, respectively. The black arrows indicate the statistically significant differences of the NF1 clinical features prevalence between the p.Arg1276 and the p.Lys1423 studied groups, and the cohort of individuals carrying one of the most frequent recurrent pathogenic nonsense variants observed in the UAB database, with up and down arrows representing an increase and a decrease of the prevalence, respectively.

[A] The UAB cohort of individuals carrying one of the most frequent *NF1* nonsense pathogenic variants, that is, c.574C>T (p.Arg192*), c.910C>T (p.Arg304*), c.1246C>T (p.Arg416*), c.1318C>T (p.Arg440*), c.1381C>T (p.Arg461*), c.2041C>T (p.Arg681*), c.2446C>T (p.Arg816*), c.3721C>T (p.Arg1241*), c.3826C>T (p.Arg1276*), c.3916C>T (p.Arg1306*), c.4084C>T (p.Arg1362*), c.4537C>T (p.Arg1513*), c.5242C>T (p.Arg1748*), c.5839C>T (p.Arg1947*), c.6709C>T (p.Arg2237*), c.7285C>T (p.Arg2429*), c.7486C>T (p.Arg2496*), c.7846C>T (p.Arg2616*). [B] In individuals ≥9 years old. [C] In individuals ≥19 years old. [D] Only malignant neoplasms, not including OPGs and neurofibromas, have been taken into account. [E] As individual was classified as having a Noonan-like phenotype when at least two of the following features were present: short stature, low set ears, hypertelorism, midface hypoplasia, webbed neck, pectus abnormality and/or pulmonic stenosis. [F] As no specific growth curves are available for Hispanic and Asian populations, Hispanic and Asian individuals were excluded as having short or normal stature. **Abbreviations: CALMs** - **c**afé-**a**u-**l**ait **m**acule**s**; **OPG** - **o**ptic **p**athway **g**lioma.

Supp. Table S19. Clinical characterization of individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276 who did not fulfill the current NIH diagnostic criteria, based on physical features, not taking family history into account.

ID [A,B]	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Lisch nodules	Neurofibromas	OPG	Specific skeletal abnormalities [C]	NIH diagnostic criteria [D]
UAB-R1175-C	c.3826C>G (p.Arg1276Gly)	F	<2	<6	-	-	-	N, UN	-	0/6
UAB-R8876	c.3826C>G (p.Arg1276Gly)	PrS	<2	<6	-	-	-	N, MRI	-	0/6
UAB-R37401FN.202	c.3826C>G (p.Arg1276Gly)	RS	<2	>5	-	-	-	N, MRI	-	1/6
UAB-R3914	c.3827G>A (p.Arg1276Gln)	RS	<2	>5	-	-	-	N, MRI	-	1/6
UAB-R40711FN.204	c.3827G>A (p.Arg1276Gln)	RS	<2	>5	-	-	-	N, UN	-	1/6
UAB-R5166	c.3826_3827delinsGA (p.Arg1276Glu)	F	2-4	>5	-	-	-	N, MRI	-	1/6
UAB-R7354	c.3827G>A (p.Arg1276Gln)	RS	2-4	>5	-	-	-	N, MRI	-	1/6
EUR-R14	c.3827G>A (p.Arg1276Gln)	RS	2-4	>5	-	-	-	N, UN	-	1/6
UAB-R7157	c.3827G>A (p.Arg1276Gln)	UN	5-8	>5	-	-	-	N, MRI	-	1/6
UAB-R1256	c.3827G>A (p.Arg1276Gln)	F	9-13	>5	-	-	-	N, MRI	-	1/6
EUR-R30	c.3827G>A (p.Arg1276Gln)	RS	14-18	<6	-	-	+	N, MRI	-	1/6
UAB-R743	c.3827G>A (p.Arg1276Gln)	NS	>26	<6	-	-	+	N, UN	-	1/6

[A] Of the 30 cases not fulfilling the NIH diagnostic criteria after excluding the family history, 12 had complete clinical information including the ophthalmological results for the presence/absence of Lisch nodules and symptomatic OPGs; [B] IDs of individuals with none of the diagnostic criteria of NF1 fulfilled were bolded; [C] Only skeletal abnormalities with sphenoid dysplasia or thinning of the long bone cortex with/without pseudarthrosis have been taken into account; [D] Only clinical features without the presence/absence of a first-degree relative that meets NIH criteria have been taken into account.

Abbreviations: F - **f**amilial; **PrS** - **pr**oven **s**poradic; **RS** - **r**eportedly **s**poradic; **NS** - **n**ot **s**pecified; **UN** - **un**known; **CALMs** - **c**afé-**a**u-**l**ait **m**acule**s**; **OPG** - **o**ptic **p**athway **g**lioma; **N, UN** - **n**o symptomatic OPG, **un**known if any asymptomatic OPG is present; **N, MRI** - **n**o symptomatic and asymptomatic OPG detected by **MRI**.

Supp. Table S20. Noonan-like features in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276.

ID [A]	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Others	Noonan-like phenotype
UAB-R37401FN.202	c.3826C>G (p.Arg1276Gly)	RS	<2	>5	-	juvenile xanthogranuloma, eczema, keratosis pilaris, pruritus	PS, WN, MH, macroglossia, upslanting palpebral fissure, LPH, macrocephaly, gross motor delay, SD
UAB-R7004	c.3827G>A (p.Arg1276Gln)	RS	<2	>5	-	-	triangular face, PT, DPF, blue irides
UAB-R6444	c.3827G>A (p.Arg1276Gln)	F	<2	>5	-	-	LSE, PA
UAB-R0522-C2	c.3827G>A (p.Arg1276Gln)	F	2-4	>5	+	-	LSE, HTL, PS
UAB-R32801FN.103	c.3827G>A (p.Arg1276Gln)	RS	2-4	>5	NS	Lisch nodules	DPF, LSE, tented upper lip, bilateral epicanthal folds, a history of mild developmental delay
UAB-R3396	c.3826C>G (p.Arg1276Gly)	PrS	5-8	>5	+	Lisch nodules	*possible* (no details provided, except for a history of developmental delay in gross motor skills)
UAB-R0522-C1	c.3827G>A (p.Arg1276Gln)	F	5-8	>5	+	-	LSE, HTL, PS
UAB-R0082	c.3827G>A (p.Arg1276Gln)	PrS	5-8	>5	+	Lisch nodules, leg length discrepancy	*possible*: SS, PS, macrocephaly
UAB-R9613	c.3827G>A (p.Arg1276Gln)	RS	5-8	>5	+	-	*possible*: SS, PA, macrocephaly
UAB-R7733	c.3827G>A (p.Arg1276Gln)	F	5-8	>5	+	depigmentation consistent with vitiligo	HTL, DPF, hypertrophic cardiomyopathy, ventricular septal defect, macrocephaly
UAB-R9893	c.3827G>A (p.Arg1276Gln)	F	5-8	>5	+	Lisch nodules, scoliosis, migraines, generalized itchiness	*possible*: coarse face, frontal bossing, macrocephaly, a history of gross motor delay, ADHD
UAB-R8484	c.3827G>A (p.Arg1276Gln)	F	5-8	>5	+	-	*possible*: SS, HTL, delayed for age
UAB-R8805	c.3827G>A (p.Arg1276Gln)	PrS	5-8	>5	+	kyphosis	HTL, DPF, PS, SS, PA
UAB-R6328	c.3827G>A (p.Arg1276Gln)	F	5-8	>5	+	scoliosis	facial dysmorphism, PA, abnormal development (neurocognitive concerns), LD, SD, ADHD
UAB-R20511FN.204 [B]	c.3827G>A (p.Arg1276Gln)	RS	5-8	>5	-	tricuspid atresia, hypoplastic right heart syndrome	DPF, high forehead, mild brachycephaly, dysplastic ears with small canals, LSE, delayed for age, gross and fine motor delays, SD
UAB-R88411FN.404	c.3827G>A (p.Arg1276Gln)	RS	5-8	>5	+	scoliosis	PT, HTL, DPF, PA, macrocephaly, a history of developmental difficulties
EUR-R18	c.3827G>A (p.Arg1276Gln)	RS	5-8	>5	+	Lisch nodules	*possible*: LPH, coarse facial features
UAB-R0843	c.3827G>A (p.Arg1276Gln)	RS	9-13	>5	-	*possible* spinal neurofibromas	WN, HTL, DPF, mild supravalvular aortic stenosis, macrocephaly, delayed for age, gross motor delay, SD, LD
UAB-R8484-S	c.3827G>A (p.Arg1276Gln)	F	9-13	>5	+	cutaneous neurofibromas, sphenoid wing dysplasia	HTL, SS, DPF, delayed for age, ADD, LD
UAB-R1256	c.3827G>A (p.Arg1276Gln)	F	9-13	>5	-	severe scoliosis	*possible*: SS, PS, developmental delay
UAB-R7596	c.3827G>A (p.Arg1276Gln)	NS	9-13	>5	+	-	SS, LSE, HTL, PA, abnormal development, LD
UAB-R3896	c.3827G>A (p.Arg1276Gln)	F	9-13	>5	+	hypertension	PT, DPF, bossed forehead, LD
EUR-R16	c.3827G>A (p.Arg1276Gln)	RS	9-13	>5	-	Lisch nodules	*possible* (no details provided)
EUR-R19	c.3827G>A (p.Arg1276Gln)	RS	9-13	>5	UN	-	*possible*: SS, DPF, delayed for age, LD
UAB-R8304	c.3827G>T (p.Arg1276Leu)	F	9-13	>5	+	scoliosis, sphenoid wing dysplasia	*possible*: SS, MH, ADD, LD

ID	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Others	Noonan-like phenotype
UAB-R0193	c.3826C>G (p.Arg1276Gly)	F	14-18	>5	+	spinal neurofibromas	*possible* (no details provided)
EUR-R8	c.3826C>G (p.Arg1276Gly)	F	14-18	>5	+	scoliosis	PT, HTL, LD
UAB-R875	c.3827G>A (p.Arg1276Gln)	RS	14-18	>5	+	aortic stenosis, renal artery stenosis, glioma other than OPG	SS, PT, WN, PA, LD, ADD
UAB-R4232	c.3827G>A (p.Arg1276Gln)	F	14-18	>5	+	supratentorial anaplastic ependymoma, seizures	HTL, LSE, drooping lids, PA, LD
UAB-R3605	c.3827G>A (p.Arg1276Gln)	PrS	14-18	>5	+	-	*possible*: LSE, midface depression
UAB-R76501FN.202 [C]	c.3827G>A (p.Arg1276Gln)	UN	14-18	>5	+	scoliosis	MPH, LPH
UAB-R4762	c.3827G>A (p.Arg1276Gln)	RS	19-26	>5	-	plexiform neurofibroma	WN, LPH, PA, macrocephaly, LD
EUR-R28-S	c.3827G>A (p.Arg1276Gln)	F	19-26	>5	+	Lisch nodules	HTL, LSE, short palpebral fissures
UAB-R7733-F	c.3827G>A (p.Arg1276Gln)	F	>26	>5	+	mild scoliosis	LSE, MH, jaw asymmetry, mild PT, macrocephaly
UAB-R5957	c.3827G>A (p.Arg1276Gln)	F	>26 †	>5	+	scoliosis, osteopenia, hypertension, astrocytoma	*possible* (no details provided), LD
EUR-R26	c.3827G>A (p.Arg1276Gln)	F	>26	>5	-	spinal neurofibromas	*possible*: PS, SS

[A] IDs of individuals with the negative RASopathy NGS panel were bolded (see details in Supporting Information Table S10). [B] This individual had a duplication of 1.2 Mb at 9p24.3p24.2 of uncertain significance detected by aCGH. [C] Mosaic case (mutant allele fraction ~31%).

Abbreviations: **NS** - **n**ot **s**pecified; **UN** - **un**known; **F** - **f**amilial; **RS** - **r**eportedly **s**poradic; **PrS** - **p**roven **s**poradic; **HTL** - **h**yper**t**e**l**orism; **WN** - short/**w**ebbed **n**eck; **MH** - **m**idface **h**ypoplasia; **LSE** - **l**ow **s**et **e**ars; **LPH** - **l**ow **p**osterior **h**airline; **SS** - **s**hort **s**tature; **PA** - **p**ectus **a**bnormality; **PT** - **pt**osis; **PS** - **p**ulmonic **s**tenosis; **DPF** - **d**ownslanting **p**alpebral **f**issures; **ADD** - **a**ttention **d**eficit **d**isorder; **ADHD** - **a**ttention **d**eficit **h**yperactivity **d**isorder; **LD** - **l**earning **d**isability; **SD** - **s**peech **d**elay; **aCGH** - **a**rray-**c**omparative **g**enomic **h**ybridization; **NGS** - **n**ext **g**eneration **s**equencing; **OPG** - **o**ptic **p**athway **g**lioma.

Supp. Table S21. Noonan-like features in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Lys1423.

ID [A]	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Others	Noonan-like phenotype
UAB-R3301	c.4267A>G (p.Lys1423Glu)	RS	<2	<6	-	-	LSE, PT, abnormal development
UAB-R2864	c.4267A>G (p.Lys1423Glu)	RS	<2	>5	+	-	SS, PT, LSE, PS, delayed for age, hypotonic
UAB-R42511FN.204	c.4267A>G (p.Lys1423Glu)	RS	<2	<6	+	-	PT, LSE, DPF
EUR-R55	c.4267A>G (p.Lys1423Glu)	PrS	<2	>5	+	-	PT, LPH, HTL, LSE
UAB-R7671	c.4267A>G (p.Lys1423Glu)	UN	2-4	>5	+	Lisch nodules, right tibial torsion	PS, PA, SD, ADHD
UAB-R8917	c.4267A>G (p.Lys1423Glu)	RS	2-4	>5	-	-	PS, HTL, LSE, MH, macrocephaly, abnormal development, LD, SD
EUR-R38	c.4267A>G (p.Lys1423Glu)	RS	2-4	>5	+	Lisch nodules, plexiform neurofibroma, sphenoid wing dysplasia	PT, HTL, ADHD
UAB-R9795	c.4268A>C (p.Lys1423Thr)	RS	2-4	>5	+	-	SS, MH, HTL, PT
UAB-R2741	c.4267A>G (p.Lys1423Glu)	PrS	5-8	>5	+	subcutaneous and spinal neurofibromas, headaches and neck pain	LSE, MH, HTL, macrocephaly, SD
UAB-R5313	c.4267A>G (p.Lys1423Glu)	PrS	5-8	>5	+	cutaneous, intradermal and subcutaneous neurofibromas	SS, LSE, HTL, ADD, ADHD, LD
UAB-R7995	c.4267A>G (p.Lys1423Glu)	RS	5-8	>5	-	-	LSE, HTL, PA
EUR-R33	c.4267A>C (p.Lys1423Gln)	F	9-13	>5	+	-	PT, HTL, LSE, delayed for age, LD
UAB-R746	c.4267A>G (p.Lys1423Glu)	RS	9-13	>5	-	plexiform, cutaneous and subcutaneous neurofibromas	SS, LPH, DPF, PT, epicanthal folds, LSE, WN, PA, abnormal development, ADHD
UAB-R0367	c.4267A>G (p.Lys1423Glu)	RS	9-13	>5	+	asymptomatic OPG, scoliosis	*possible*: SS, PS
UAB-R84111FN.204	c.4267A>G (p.Lys1423Glu)	RS	9-13	>5	+	asymptomatic OPG, scoliosis, hypothalamic glioma	*possible*: PA, DPF, delayed for age
UAB-R5651	c.4267A>G (p.Lys1423Glu)	F	9-13	>5	+	-	LSE, HTL, WN, PS, PT, PA, ventricular septal defect, macrocephaly, gross and fine motor delays
EUR-R40	c.4267A>G (p.Lys1423Glu)	F	9-13	>5	+	-	PT, HTL, DPF, macrocephaly, LD
EUR-R54-C	c.4267A>G (p.Lys1423Glu)	F	9-13	>5	-	-	high and large forehead, telecanthus, bilateral epicanthus, PT, short nose, posteriorly rotated ears, PA, fine motor delay, ADD, ADHD
UAB-R5042	c.4267A>G (p.Lys1423Glu)	RS	14-18	>5	+	bicuspid aortic valve, aortic regurgitation, aortic root dilatation, parachute deformity of mitral valve	LSE, PT, PA, LD
UAB-R7403	c.4267A>G (p.Lys1423Glu)	RS	14-18	>5	+	plexiform neurofibroma	LSE, MH, HTL, PS, ADD
UAB-R2551	c.4267A>G (p.Lys1423Glu)	RS	14-18	>5	+	plexiform neurofibromas, dysplastic vertebrae, lordosis, kyphosis, leg pain, seizures, a mild tremor	PS, broad nose, malocclusion of the teeth, DPF, WN with a bulbous tip to the nose, mild mental retardation, ADD
EUR-R42	c.4267A>G (p.Lys1423Glu)	RS	14-18	>5	+	Lisch nodules	PS, HTL, LSE, PT, DPF, ADHD, LD

ID [A]	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Others	Noonan-like phenotype
UAB-R1185	c.4267A>G (p.Lys1423Glu)	PrS	19-26	>5	-	cutaneous and spinal neurofibromas, pilocytic astrocytoma, non-ossifying fibromas	*possible*: PT, abnormal ear shape
UAB-R4735	c.4267A>G (p.Lys1423Glu)	RS	>26	>5	+	cutaneous neurofibromas, bone cysts, non-ossifying fibromas, multiple giant cell tumors, a history of rhabdomyosarcoma	SS, LSE, HTL, macrocephaly, asymmetry secondary to the deformation of the giant cell tumor located in jaw
UAB-R97511FN.204	c.4267A>G (p.Lys1423Glu)	NS	>26	>5	+	cutaneous and spinal neurofibromas, brainstem glioma	LSE, DPF, macrocephaly
EUR-R43	c.4267A>G (p.Lys1423Glu)	RS	>26	>5	+	Lisch nodules, cutaneous and subcutaneous neurofibromas	PS, PT
EUR-R45	c.4267A>G (p.Lys1423Glu)	RS	>26	>5	+	Lisch nodules, cutaneous and subcutaneous neurofibromas, meningioma, colon adenocarcinoma, motor polyneuropathy with myelin damage (bilateral feet steppage)	PT, HTL

[A] IDs of individuals with the negative RASopathy NGS panel were bolded, except for two individuals (EUR-R42 and EUR-R43) tested only for *PTPN11* (see details in Supporting Information Table S12).

Abbreviations: NS - **n**ot **s**pecified; **UN** - **un**known; **F** - **f**amilial; **RS** - **r**eportedly **s**poradic; **PrS** - **p**roven **s**poradic; **HTL** - **h**yper**t**e**l**orism; **WN** - short/**w**ebbed **n**eck; **MH** - **m**idface **h**ypoplasia; **LSE** - **l**ow **s**et **e**ars; **LPH** - **l**ow **p**osterior **h**airline; **SS** - **s**hort **s**tature; **PA** - **p**ectus **a**bnormality; **PT** - **pt**osis; **PS** - **p**ulmonic **s**tenosis; **DPF** - **d**ownslanting **p**alpebral **f**issures; **ADD** - **a**ttention **d**eficit **d**isorder; **ADHD** - **a**ttention **d**eficit **h**yperactivity **d**isorder; **LD** - **l**earning **d**isability; **SD** - **s**peech **d**elay; **aCGH** - **a**rray-**c**omparative **g**enomic **h**ybridization; **NGS** - **n**ext **g**eneration **s**equencing; **OPG** - **o**ptic **p**athway **g**lioma.

Supp. Table S22. Cardiovascular abnormalities in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276.

ID	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Others	Cardiovascular abnormalities	Noonan-like phenotype
UAB-R37401FN.202	c.3826C>G (p.Arg1276Gly)	RS	<2	>5	-	juvenile xanthogranuloma, macrocephaly, gross motor delay, SD	mild pulmonic stenosis	+
UAB-R0522-C2	c.3827G>A (p.Arg1276Gln)	F	2-4	>5	+	-	pulmonic stenosis	+
UAB-R6328-S	c.3827G>A (p.Arg1276Gln)	F	5-8	>5	-	macrocephaly	pulmonic stenosis	-
UAB-R853	c.3827G>A (p.Arg1276Gln)	RS	5-8	>5	+	SS, motor delay	Moya moya disease, left middle artery stenosis	-
UAB-R0522-C1	c.3827G>A (p.Arg1276Gln)	F	5-8	>5	+	-	pulmonic stenosis	+
UAB-R0082	c.3827G>A (p.Arg1276Gln)	PrS	5-8	>5	+	Lisch nodules, leg length discrepancy, SS, macrocephaly	pulmonic stenosis	-
UAB-R7733	c.3827G>A (p.Arg1276Gln)	F	5-8	>5	+	macrocephaly	hypertrophic cardiomyopathy, ventricular septal defect	+
UAB-R9514	c.3827G>A (p.Arg1276Gln)	RS	5-8	>5	-	Lisch nodules, macrocephaly	mild supravalvular pulmonic pulmonic and aortic stenosis	-
UAB-R8805	c.3827G>A (p.Arg1276Gln)	PrS	5-8	>5	+	pectus excavatum, kyphosis	pulmonic stenosis	+
UAB-R20511FN.204	c.3827G>A (p.Arg1276Gln)	RS	5-8	>5	-	delayed for age, gross and fine motor delays, SD	tricuspid atresia, hypoplastic right heart syndrome	+
UAB-R0843	c.3827G>A (p.Arg1276Gln)	RS	9-13	>5	-	macrocephaly, delayed for age, gross motor delay, SD, LD	mild supravalvular aortic stenosis	+
UAB-R1256	c.3827G>A (p.Arg1276Gln)	F	9-13	>5	-	severe scoliosis, SS, developmental delay	left ventricular hypertrophy, aortic stenosis, aortic septal defect, pulmonic stenosis	-
UAB-R3896	c.3827G>A (p.Arg1276Gln)	F	9-13	>5	+	LD	hypertension	+
UAB-R875	c.3827G>A (p.Arg1276Gln)	F	14-18	>5	+	scoliosis, pectus excavatum, other glioma than OPG, LD, ADD	aortic stenosis, renal artery stenosis, pulmonic valve stenosis	+
EUR-R7	c.3826C>G (p.Arg1276Gly)	RS	>26	>5	UN	6-99 cutaneous neurofibromas	myocardial infarction	UN
UAB-R1256-M	c.3827G>A (p.Arg1276Gln)	F	>26	>5	+	2-6 cutaneous, subcutaneous, intradermal and spinal neurofibromas, scoliosis	aortic septal defect	-
EUR-R21-F	c.3827G>A (p.Arg1276Gln)	RS	>26	>5	+	6-99 subcutaneous and spinal neurofibromas, colon cancer	hypertension	-
EUR-R23	c.3827G>A (p.Arg1276Gln)	RS	>26	>5	-	plexiform, subcutaneous and spinal neurofibromas, scoliosis	hypertension	-
EUR-R24	c.3827G>A (p.Arg1276Gln)	RS	>26	>5	-	Lisch nodules, plexiform, cutaneous, subcutaneous and spinal neurofibromas	pulmonic stenosis	-
EUR-R26	c.3827G>A (p.Arg1276Gln)	F	>26	>5	-	spinal neurofibromas, scoliosis, osteopenia	hypertension, pulmonic stenosis	-
UAB-R3393-F	c.3827G>T (p.Arg1276Leu)	RS	>26	>5	+	-	hypertension, carotid artery stenosis, coronary disease, strokes	UN
UAB-R37411FN.204-F	c.3826C>G (p.Arg1276Gly)	UN	>26	>5	+	2-6 cutaneous neurofibromas, lipoma, diffuse astrocytoma on right frontal lobe (WHO grade I)	atrial fibrillation	NS

Abbreviations: UN - **un**known; **F** - **f**amilial; **RS** - **r**eportedly **s**poradic; **PrS** - **p**roven **s**poradic; **SS** - **s**hort **s**tature; **ADD** - **a**ttention **d**eficit **d**isorder; **LD** - **l**earning **d**isability; **SD** - **s**peech **d**elay; **SS** - **s**hort **s**tature**; OPG** - **o**ptic **p**athway **g**lioma; **NS** - **n**ot **s**pecified.

Supp. Table S23. Cardiovascular abnormalities in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Lys1423.

ID	Pathogenic variant	Family history	Age, years	CALMs	Freckles	Others	Cardiovascular abnormalities	Noonan-like phenotype
UAB-R1816	c.4267A>C (p.Lys1423Gln)	UN	<2	>5	+	gross motor delay	pulmonic stenosis	-
UAB-R2864	c.4267A>G (p.Lys1423Glu)	RS	<2	>5	+	Lisch nodules, SS, left midbrain lesion, likely low-grade glioma, delayed for age, hypotonic	pulmonic stenosis	+
UAB-R3936	c.4267A>G (p.Lys1423Glu)	F	<2	<6	+	-	pulmonic stenosis	-
UAB-R7671	c.4267A>G (p.Lys1423Glu)	UN	2-4	>5	+	Lisch nodules, right tibial torsion, mild pectus carinatum, SD, ADHD	pulmonic stenosis	+
UAB-R8917	c.4267A>G (p.Lys1423Glu)	RS	2-4	>5	-	macrocephaly, abnormal development, LD, SD	pulmonic stenosis	+
UAB-R6953	c.4267A>G (p.Lys1423Glu)	NS	5-8	NS	NS	SD, LD	a history of small ventricular septal defect and functional murmur	NS
UAB-R2305	c.4267A>G (p.Lys1423Glu)	RS	9-13	>5	-	asymptomatic OPG	aortic stenosis	-
UAB-R0367	c.4267A>G (p.Lys1423Glu)	RS	9-13	>5	+	asymptomatic OPG, scoliosis, SS	pulmonic stenosis	-
UAB-R5651	c.4267A>G (p.Lys1423Glu)	F	9-13	>5	+	pectus excavatum, pes planus, macrocephaly, gross and fine motor delays	ventricular septal defect, pulmonic valve stenosis	+
UAB-R5042	c.4267A>G (p.Lys1423Glu)	RS	14-18	>5	+	pectus excavatum, LD	bicuspid aortic valve, aortic regurgitation, mild aortic root dilatation, parachute deformity of mitral valve	+
UAB-R7403	c.4267A>G (p.Lys1423Glu)	RS	14-18	>5	+	plexiform neurofibroma, ADD	pulmonic stenosis	+
UAB-R4165	c.4267A>G (p.Lys1423Glu)	UN	14-18	>5	+	Lisch nodules, abnormal development, LD	Wolff-Parkinson-White syndrome	-
UAB-R2551	c.4267A>G (p.Lys1423Glu)	RS	14-18	>5	+	plexiform neurofibroma, dysplastic vertebra, lumbar lordosis and slight cervical kyphosis visible on X-ray imaging, mild mental retardation, ADD, dyspraxia	pulmonic stenosis	+
EUR-R42	c.4267A>G (p.Lys1423Glu)	RS	14-18	>5	+	Lisch nodules, ADHD, LD	pulmonic stenosis	+
UAB-R9485	c.4267A>G (p.Lys1423Glu)	RS	19-26	>5	+	a history of scoliosis and mild kyphosis visible on X-ray imaging, multiple non-ossifying fibromas, LD	a history of hypertension	-
UAB-R4031	c.4267A>G (p.Lys1423Glu)	RS	>26	>5	+	Lisch nodules, >500 cutaneous and subcutaneous neurofibroma, osteoporosis, scoliosis, SS, macrocephaly	dilated ventricles	-
UAB-R4031-C	c.4267A>G (p.Lys1423Glu)	F	>26	>5	+	100-500 cutaneous, 6-99 subcutaneous and 100-500 intradermal neurofibromas, dural ectasia in thoracic spine, macrocephaly	Moya moya disease	-
UAB-R4031	c.4267A>G (p.Lys1423Glu)	RS	>26	>5	+	Lisch nodules, >500 cutaneous and subcutaneous neurofibromas, osteoporosis, scoliosis, SS, macrocephaly	dilated ventricles	-
EUR-R43	c.4267A>G (p.Lys1423Glu)	RS	>26	>5	+	Lisch nodules, cutaneous and subcutaneous neurofibromas (# unknown)	pulmonic valve stenosis, hypertension	+
EUR-R52	c.4267A>G (p.Lys1423Glu)	RS	>26	>5	-	Lisch nodules, 100-500 cutaneous, 6-99 subcutaneous and 6-99 intradermal neurofibromas, cubitus valgus, SS, hypothalamic glioma	mitral valve anomaly	-

Abbreviations: NS - **n**ot **s**pecified; **UN** - **un**known; **F** - **f**amilial; **RS** - **r**eportedly **s**poradic; **PrS** - **p**roven **s**poradic; **SS** - **s**hort **s**tature; **ADD** - **a**ttention **d**eficit **d**isorder; **ADHD** - **a**ttention **d**eficit **h**yperactivity **d**isorder; **LD** - **l**earning **d**isability; **SD** - **s**peech **d**elay; **OPG** - **o**ptic **p**athway **g**lioma.

Supp. Table S24. List of all adjusted *P* values from Table 1 after applying the Benjamini-Hochberg correction for multiple testing with false discovery rates (FDR) at 0.05 and 0.01.

***P* value**	**index**	**Statistical significance at FDR=0.05**		**Statistical significance at FDR=0.01**	
		B-H critical [A]	**value** [B]	**B-H critical** [A]	**value** [B]
1.0000	1	0.0250		0.0050	
1.0000	2	0.0247		0.0049	
1.0000	3	0.0243		0.0049	
1.0000	4	0.0240		0.0048	
1.0000	5	0.0236		0.0047	
1.0000	6	0.0233		0.0047	
1.0000	7	0.0229		0.0046	
1.0000	8	0.0226		0.0045	
1.0000	9	0.0222		0.0044	
1.0000	10	0.0219		0.0044	
1.0000	11	0.0215		0.0043	
1.0000	12	0.0212		0.0042	
1.0000	13	0.0208		0.0042	
1.0000	14	0.0205		0.0041	
1.0000	15	0.0201		0.0040	
1.0000	16	0.0198		0.0040	
1.0000	17	0.0194		0.0039	
1.0000	18	0.0191		0.0038	
1.0000	19	0.0188		0.0038	
1.0000	20	0.0184		0.0037	
1.0000	21	0.0181		0.0036	
1.0000	22	0.0177		0.0035	
0.8698	23	0.0174		0.0035	
0.8138	24	0.0170		0.0034	
0.7909	25	0.0167		0.0033	
0.7810	26	0.0163		0.0033	
0.7660	27	0.0160		0.0032	
0.7613	28	0.0156		0.0031	
0.6633	29	0.0153		0.0031	
0.6237	30	0.0149		0.0030	
0.5911	31	0.0146		0.0029	
0.5809	32	0.0142		0.0028	
0.5711	33	0.0139		0.0028	
0.4645	34	0.0135		0.0027	
0.4498	35	0.0132		0.0026	
0.4368	36	0.0128		0.0026	
0.3860	37	0.0125		0.0025	
0.2612	38	0.0122		0.0024	
0.2579	39	0.0118		0.0024	
0.2485	40	0.0115		0.0023	
0.2473	41	0.0111		0.0022	
0.2430	42	0.0108		0.0022	
0.2411	43	0.0104		0.0021	
0.2359	44	0.0101		0.0020	
0.2259	45	0.0097		0.0019	
0.1722	46	0.0094		0.0019	
0.1676	47	0.0090		0.0018	
0.1466	48	0.0087		0.0017	
0.1319	49	0.0083		0.0017	
0.1168	50	0.0080		0.0016	
0.0810	51	0.0076		0.0015	
0.0675	52	0.0073		0.0015	
0.0585	53	0.0069		0.0014	
0.0520	54	0.0066		0.0013	
0.0196	55	0.0063		0.0013	
0.0195	56	0.0059		0.0012	
0.0120	57	0.0056		0.0011	
0.0103	58	0.0052		0.0010	
0.0085	59	0.0049		0.0010	
0.0023	60	0.0045	*	0.0009	
0.0023	61	0.0042	*	0.0008	
0.0005	62	0.0038	*	0.0008	*
0.0001	63	0.0035	*	0.0007	*
0.0001	64	0.0031	*	0.0006	*
0.0001	65	0.0028	*	0.0006	*
0.0001	66	0.0024	*	0.0005	*
0.0001	67	0.0021	*	0.0004	*
0.0001	68	0.0017	*	0.0003	*
0.0001	69	0.0014	*	0.0003	*
0.0001	70	0.0010	*	0.0002	*
0.0001	71	0.0007	*	0.0001	*
0.0001	72	0.0003	*	0.0001	*

[A] According to Thissen et al. (2002); [B] An asterisk (*) indicates the statistically significant *P*-value after B-H correction.

Supp. Table S25. List of all adjusted *P* values from Table 2 after applying the Benjamini-Hochberg correction for multiple testing with false discovery rates (FDR) at 0.05 and 0.01.

***P* value**	**index**	**Statistical significance at FDR=0.05**		**Statistical significance at FDR=0.01**	
		B-H critical [A]	**value** [B]	**B-H critical** [A]	**value** [B]
1.0000	1	0.0250		0.0050	
1.0000	2	0.0247		0.0049	
1.0000	3	0.0243		0.0049	
1.0000	4	0.0240		0.0048	
1.0000	5	0.0236		0.0047	
1.0000	6	0.0233		0.0047	
1.0000	7	0.0229		0.0046	
0.9128	8	0.0226		0.0045	
0.8900	9	0.0222		0.0044	
0.8303	10	0.0219		0.0044	
0.8290	11	0.0215		0.0043	
0.7607	12	0.0212		0.0042	
0.7477	13	0.0208		0.0042	
0.7450	14	0.0205		0.0041	
0.7041	15	0.0201		0.0040	
0.6940	16	0.0198		0.0040	
0.6804	17	0.0194		0.0039	
0.5452	18	0.0191		0.0038	
0.5236	19	0.0188		0.0038	
0.5224	20	0.0184		0.0037	
0.4853	21	0.0181		0.0036	
0.4514	22	0.0177		0.0035	
0.3383	23	0.0174		0.0035	
0.3162	24	0.0170		0.0034	
0.3039	25	0.0167		0.0033	
0.2560	26	0.0163		0.0033	
0.2461	27	0.0160		0.0032	
0.2459	28	0.0156		0.0031	
0.2028	29	0.0153		0.0031	
0.1998	30	0.0149		0.0030	
0.1895	31	0.0146		0.0029	
0.1685	32	0.0142		0.0028	
0.1666	33	0.0139		0.0028	
0.1031	34	0.0135		0.0027	
0.0867	35	0.0132		0.0026	
0.0844	36	0.0128		0.0026	
0.0817	37	0.0125		0.0025	
0.0748	38	0.0122		0.0024	
0.0549	39	0.0118		0.0024	
0.0507	40	0.0115		0.0023	
0.0505	41	0.0111		0.0022	
0.0467	42	0.0108		0.0022	
0.0371	43	0.0104		0.0021	
0.0203	44	0.0101		0.0020	
0.0104	45	0.0097		0.0019	
0.0076	46	0.0094	*	0.0019	
0.0070	47	0.0090	*	0.0018	
0.0070	48	0.0087	*	0.0017	
0.0059	49	0.0083	*	0.0017	
0.0055	50	0.0080	*	0.0016	
0.0040	51	0.0076	*	0.0015	
0.0038	52	0.0073	*	0.0015	
0.0037	53	0.0069	*	0.0014	
0.0034	54	0.0066	*	0.0013	
0.0016	55	0.0063	*	0.0013	
0.0012	56	0.0059	*	0.0012	
0.0002	57	0.0056	*	0.0011	*
0.0001	58	0.0052	*	0.0010	*
0.0001	59	0.0049	*	0.0010	*
0.0001	60	0.0045	*	0.0009	*
0.0001	61	0.0042	*	0.0008	*
0.0001	62	0.0038	*	0.0008	*
0.0001	63	0.0035	*	0.0007	*
0.0001	64	0.0031	*	0.0006	*
0.0001	65	0.0028	*	0.0006	*
0.0001	66	0.0024	*	0.0005	*
0.0001	67	0.0021	*	0.0004	*
0.0001	68	0.0017	*	0.0003	*
0.0001	69	0.0014	*	0.0003	*
0.0001	70	0.0010	*	0.0002	*
0.0001	71	0.0007	*	0.0001	*
0.0001	72	0.0003	*	0.0001	*

[A] According to Thissen et al. (2002); [B] An asterisk (*) indicates the statistically significant *P*-value after B-H correction.

Supp. Table S26. List of all adjusted *P* values from Table 3 after applying the Benjamini-Hochberg correction for multiple testing with false discovery rates (FDR) at 0.05 and 0.01.

***P* value**	**index**	**Statistical significance at FDR=0.05**		**Statistical significance at FDR=0.01**	
		B-H critical [A]	**value** [B]	**B-H critical** [A]	**value** [B]
1.0000	1	0.0250		0.0050	
1.0000	2	0.0247		0.0049	
1.0000	3	0.0243		0.0049	
1.0000	4	0.0240		0.0048	
0.8102	5	0.0236		0.0047	
0.8085	6	0.0233		0.0047	
0.7941	7	0.0229		0.0046	
0.7672	8	0.0226		0.0045	
0.6357	9	0.0222		0.0044	
0.6305	10	0.0219		0.0044	
0.5436	11	0.0215		0.0043	
0.53*56	12	0.0212		0.0042	
0.5239	13	0.0208		0.0042	
0.5071	14	0.0205		0.0041	
0.4671	15	0.0201		0.0040	
0.3489	16	0.0198		0.0040	
0.3474	17	0.0194		0.0039	
0.3457	18	0.0191		0.0038	
0.3457	19	0.0188		0.0038	
0.3033	20	0.0184		0.0037	
0.2758	21	0.0181		0.0036	
0.2705	22	0.0177		0.0035	
0.2696	23	0.0174		0.0035	
0.2552	24	0.0170		0.0034	
0.2522	25	0.0167		0.0033	
0.2184	26	0.0163		0.0033	
0.2141	27	0.0160		0.0032	
0.1851	28	0.0156		0.0031	
0.1771	29	0.0153		0.0031	
0.1522	30	0.0149		0.0030	
0.1494	31	0.0146		0.0029	
0.1168	32	0.0142		0.0028	
0.1148	33	0.0139		0.0028	
0.0955	34	0.0135		0.0027	
0.0908	35	0.0132		0.0026	
0.0863	36	0.0128		0.0026	
0.0847	37	0.0125		0.0025	
0.0695	38	0.0122		0.0024	
0.0694	39	0.0118		0.0024	
0.0571	40	0.0115		0.0023	
0.0358	41	0.0111		0.0022	
0.0306	42	0.0108		0.0022	
0.0257	43	0.0104		0.0021	
0.0194	44	0.0101		0.0020	
0.0189	45	0.0097		0.0019	
0.0176	46	0.0094		0.0019	
0.0173	47	0.0090		0.0018	
0.0097	48	0.0087		0.0017	
0.0074	49	0.0083	*	0.0017	
0.0070	50	0.0080	*	0.0016	
0.0053	51	0.0076	*	0.0015	
0.0052	52	0.0073	*	0.0015	
0.0022	53	0.0069	*	0.0014	
0.0011	54	0.0066	*	0.0013	*
0.0010	55	0.0063	*	0.0013	*
0.0002	56	0.0059	*	0.0012	*
0.0002	57	0.0056	*	0.0011	*
0.0001	58	0.0052	*	0.0010	*
0.0001	59	0.0049	*	0.0010	*
0.0001	60	0.0045	*	0.0009	*
0.0001	61	0.0042	*	0.0008	*
0.0001	62	0.0038	*	0.0008	*
0.0001	63	0.0035	*	0.0007	*
0.0001	64	0.0031	*	0.0006	*
0.0001	65	0.0028	*	0.0006	*
0.0001	66	0.0024	*	0.0005	*
0.0001	67	0.0021	*	0.0004	*
0.0001	68	0.0017	*	0.0003	*
0.0001	69	0.0014	*	0.0003	*
0.0001	70	0.0010	*	0.0002	*
0.0001	71	0.0007	*	0.0001	*
0.0001	72	0.0003	*	0.0001	*

[A] According to Thissen et al. (2002); [B] An asterisk (*) indicates the statistically significant *P*-value after B-H correction.

Supp. Table S27. List of all adjusted *P* values from Supporting Information Table S18 after applying the Benjamini-Hochberg correction for multiple testing with false discovery rates (FDR) at 0.05 and 0.01.

***P* value**	**index**	**Statistical significance at FDR=0.05**		**Statistical significance at FDR=0.01**	
		B-H critical [A]	**value** [B]	**B-H critical** [A]	**value** [B]
1.0000	1	0.0250		0.0050	
1.0000	2	0.0245		0.0049	
1.0000	3	0.0241		0.0048	
1.0000	4	0.0236		0.0047	
1.0000	5	0.0231		0.0046	
1.0000	6	0.0227		0.0045	
0.8689	7	0.0222		0.0044	
0.8678	8	0.0218		0.0044	
0.8463	9	0.0213		0.0043	
0.7801	10	0.0208		0.0042	
0.7787	11	0.0204		0.0041	
0.7707	12	0.0199		0.0040	
0.6799	13	0.0194		0.0039	
0.6526	14	0.0190		0.0038	
0.5876	15	0.0185		0.0037	
0.5797	16	0.0181		0.0036	
0.5167	17	0.0176		0.0035	
0.4327	18	0.0171		0.0034	
0.3243	19	0.0167		0.0033	
0.3145	20	0.0162		0.0032	
0.3006	21	0.0157		0.0031	
0.2667	22	0.0153		0.0031	
0.2326	23	0.0148		0.0030	
0.2251	24	0.0144		0.0029	
0.2210	25	0.0139		0.0028	
0.2200	26	0.0134		0.0027	
0.1689	27	0.0130		0.0026	
0.1183	28	0.0125		0.0025	
0.1146	29	0.0120		0.0024	
0.0894	30	0.0116		0.0023	
0.0436	31	0.0111		0.0022	
0.0109	32	0.0106		0.0021	
0.0091	33	0.0102	*****	0.0020	
0.0089	34	0.0097	*****	0.0019	
0.0083	35	0.0093	*	0.0019	
0.0044	36	0.0088	*	0.0018	
0.0043	37	0.0083	*	0.0017	
0.0038	38	0.0079	*	0.0016	
0.0033	39	0.0074	*	0.0015	
0.0024	40	0.0069	*	0.0014	
0.0008	41	0.0065	*	0.0013	*
0.0006	42	0.0060	*	0.0012	*
0.0006	43	0.0056	*	0.0011	*
0.0002	44	0.0051	*	0.0010	*
0.0001	45	0.0046	*	0.0009	*
0.0001	46	0.0042	*	0.0008	*
0.0001	47	0.0037	*	0.0007	*
0.0001	48	0.0032	*	0.0006	*
0.0001	49	0.0028	*	0.0006	*
0.0001	50	0.0023	*	0.0005	*
0.0001	51	0.0019	*	0.0004	*
0.0001	52	0.0014	*	0.0003	*
0.0001	53	0.0009	*****	0.0002	*
0.0001	54	0.0005	*****	0.0001	*****

[A] According to Thissen et al. (2002); [B] An asterisk (*) indicates the statistically significant *P*-value after B-H correction.

Supp. Table S28. Skeletal abnormalities in individuals heterozygous for pathogenic *NF1* missense variants affecting p.Met1149, p.Arg1276 and p.Lys1423.

Skeletal abnormality	N (%)		
	p.Met1149	**p.Arg1276**	**p.Lys1423**
Pectus excavatum/carinatum	8/61 (13.1)	13/100 (13)	10/83 (12.1)
Scoliosis	3/61 (4.9)	18/100 (18)	17/83 (20.5)
Sphenoid wing dysplasia	1/61 (1.6)	2/100 (2)	1/83 (1.2)
Other	marfanoid habitus, genu valgum, leg length discrepancy, clinodactyly and exostosis	marfanoid habitus, leg or limb length discrepancy, kyphosis, cherubism, osteoporosis	pes planus, kyphosis, bone cysts, lordosis, long bone dysplasia, cubitus valgus, osteoporosis, dysplastic vertebrae, pseudoarthrosis
Total	15/61 (24.6)	32/100 (32)	34/83 (41)

Supp. Table S29. Comparison of clinical features of the cohorts of individuals heterozygous for pathogenic *NF1* missense variants affecting p.Met1149, p.Arg1276 and p.Lys1423 referred for molecular *NF1* genetic testing to the Medical Genomics Laboratory at the University of Alabama at Birmingham (UAB) and to the collaborating institutions in Europe (EUR).

	N (%)						*P* value (2-tailed Fisher's exact test)		
	p.Met1149 (UAB) [A]	**p.Met1149 (EUR)**	**p.Arg1276 (UAB)**	**p.Arg1276 (EUR)**	**p.Lys1423 (UAB) [A]**	**p.Lys1423 (EUR)**	**p.Met1149 (UAB versus EUR)**	**p.Arg1276 (UAB versus EUR)**	**p.Lys1423 (UAB versus EUR)**
>5 CALMs	56/63 (88.9)	6/6 (100)	83/90 (92.2)	28/29 (96.6)	59/63 (93.7)	27/28 (96.4)	1.0000	0.6777	1.0000
Skinfold freckling	36/60 (60)	4/5 (80)	59/87 (67.8)	15/25 (60)	43/59 (72.9)	22/26 (84.6)	0.6412	0.4808	0.2808
Lisch nodules [B]	3/27 (11.1)	0/2 (0)	5/23 (21.7)	7/16 (43.8)	11/21 (52.4)	14/16 (87.5)	1.0000	0.1743	**0.0352** [G]
Major external plexiform neurofibromas [B]	0/38 (0)	0/4 (0)	1/41 (2.4)	4/23 (17.4)	10/30 (33.3)	5/18 (27.8)	1.0000	0.0520	0.7571
Cutaneous neurofibromas [C]	0-3/22 (0-13.6)	0/2 (0)	7/23 (30.4)	7/17 (41.2)	13/15 (86.7)	10/13 (76.9)	1.0000	0.5206	0.6389
Subcutaneous neurofibromas [C]	0-3/20 (0-9.7)	0/2 (0)	11/22 (50)	10/15 (66.7)	6/12 (50)	7/11 (63.6)	1.0000	0.5000	0.6802
Symptomatic spinal neurofibromas [C]	0/19 (0)	0/2 (0)	8/19 (43)	9/17 (52.9)	0/13 (0)	1/9 (11.1)	1.0000	0.7388	0.4091
Symptomatic OPGs	0/52 (0)	0/6 (0)	0/70 (0)	0/27 (0)	0/49 (0)	1/25 (4)	1.0000	1.0000	0.3378
Asymptomatic OPGs	0/19 (0)	0/4 (0)	1/29 (3.5)	0/19 (0)	3/20 (15)	3/20 (15)	1.0000	1.0000	1.0000
Other malignant neoplasms [D]	0/51 (0)	0/6 (0)	2/67 (3)	2/27 (7.4)	4/53 (7.6)	3/24 (12.5)	1.0000	0.5759	0.6706
Skeletal abnormalities	13/55 (23.6)	2/6 (33.3)	26/74 (35.1)	6/26 (23.1)	21/59 (35.6)	13/24 (54.2)	0.6297	0.3315	0.1437
Scoliosis [C]	1/18 (3.9)	1/2 (50)	5/19 (26.3)	3/16 (18.8)	5/15 (33.3)	5/12 (41.7)	0.1947	0.7003	0.7063
Cognitive impairment and/or learning disabilities	28/60 (46.7)	3/6 (50)	39/80 (48.8)	7/25 (28)	26/60 (43.3)	10/27 (37)	1.0000	0.1050	0.6431
Noonan-like phenotype [E]	15/56 (26.8)	3/6 (50)	20/81 (24.7)	2/25 (8)	16/58 (27.6)	8/25 (32)	0.3442	0.0926	0.7929
Short stature [F]	5/30 (16.7)	0/3 (0)	11/60 (18.3)	3/20 (15)	11/31 (35.5)	10/20 (50)	1.0000	1.0000	0.3863
Macrocephaly	17/41 (41.5)	2/4 (50)	21/63 (33.3)	3/13 (23.1)	12/36 (33.3)	3/15 (20)	1.0000	0.7440	0.5034
Pulmonic stenosis	2/46 (4.4)	0/6 (0)	9/69 (13)	2/23 (8.7)	9/50 (18)	2/26 (7.7)	1.0000	0.7245	0.3130
Cardiovascular abnormalities	4/46 (8.7)	1/6 (16.7)	17/69 (24.6)	5/23 (21.7)	16/50 (32)	3/26 (11.5)	0.4726	1.0000	0.0570

[A] Excluding UAB-R1495 and UAB-R1753 carrying c.3445A>T (p.Met1149Leu) and c.4268A>T (p.Lys1423Met), respectively as the interpretation of these specific variants is "*variant of uncertain significanc*e" and "*likely pathogenic*", respectively according to the ACMG recommendations (Richards et al., 2015); [B] In individuals ≥9 years old. [C] In individuals ≥19 years old. [D] Only malignant neoplasms, not including OPGs and neurofibromas, have been taken into account. [E] As individual was classified as having a Noonan-like phenotype when at least two of the following features were present: short stature, low set ears, hypertelorism, midface hypoplasia, webbed neck, pectus abnormality and/or pulmonic stenosis. [E] As no specific growth curves are available for Hispanic and Asian populations, Hispanic and Asian individuals were excluded as having short or normal stature. [G] After applying the Benjamini-Hochberg correction $P = 0.0352$ did not remain statistically significant at FDR of 0.05 and 0.01 (see details in Supporting Information Table S30).

Abbreviations: CALMs - **c**afé-**a**u-**l**ait **m**acule**s**; **OPG** - **o**ptic **p**athway **g**lioma.

Supp. Table S30. List of all adjusted *P* values from Supporting Information Table S29 after applying the Benjamini-Hochberg correction for multiple testing with false discovery rates (FDR) at 0.05 and 0.01.

***P* value**	**index**	**Statistical significance at FDR=0.05**		**Statistical significance at FDR=0.01**	
		B-H critical [A]	**value** [B]	**B-H critical** [A]	**value** [B]
1.0000	1	0.0250		0.0050	
1.0000	2	0.0245		0.0049	
1.0000	3	0.0241		0.0048	
1.0000	4	0.0236		0.0047	
1.0000	5	0.0231		0.0046	
1.0000	6	0.0227		0.0045	
1.0000	7	0.0222		0.0044	
1.0000	8	0.0218		0.0044	
1.0000	9	0.0213		0.0043	
1.0000	10	0.0208		0.0042	
1.0000	11	0.0204		0.0041	
1.0000	12	0.0199		0.0040	
1.0000	13	0.0194		0.0039	
1.0000	14	0.0190		0.0038	
1.0000	15	0.0185		0.0037	
1.0000	16	0.0181		0.0036	
1.0000	17	0.0176		0.0035	
1.0000	18	0.0171		0.0034	
1.0000	19	0.0167		0.0033	
0.7929	20	0.0162		0.0032	
0.7571	21	0.0157		0.0031	
0.7440	22	0.0153		0.0031	
0.7388	23	0.0148		0.0030	
0.7245	24	0.0144		0.0029	
0.7063	25	0.0139		0.0028	
0.7003	26	0.0134		0.0027	
0.6802	27	0.0130		0.0026	
0.6777	28	0.0125		0.0025	
0.6706	29	0.0120		0.0024	
0.6431	30	0.0116		0.0023	
0.6412	31	0.0111		0.0022	
0.6389	32	0.0106		0.0021	
0.6297	33	0.0102		0.0020	
0.5759	34	0.0097		0.0019	
0.5206	35	0.0093		0.0019	
0.5034	36	0.0088		0.0018	
0.5000	37	0.0083		0.0017	
0.4808	38	0.0079		0.0016	
0.4726	39	0.0074		0.0015	
0.4091	40	0.0069		0.0014	
0.3863	41	0.0065		0.0013	
0.3442	42	0.0060		0.0012	
0.3378	43	0.0056		0.0011	
0.3315	44	0.0051		0.0010	
0.3130	45	0.0046		0.0009	
0.2808	46	0.0042		0.0008	
0.1947	47	0.0037		0.0007	
0.1743	48	0.0032		0.0006	
0.1437	49	0.0028		0.0006	
0.1050	50	0.0023		0.0005	
0.0926	51	0.0019		0.0004	
0.0570	52	0.0014		0.0003	
0.0520	53	0.0009		0.0002	
0.0352	54	0.0005		0.0001	

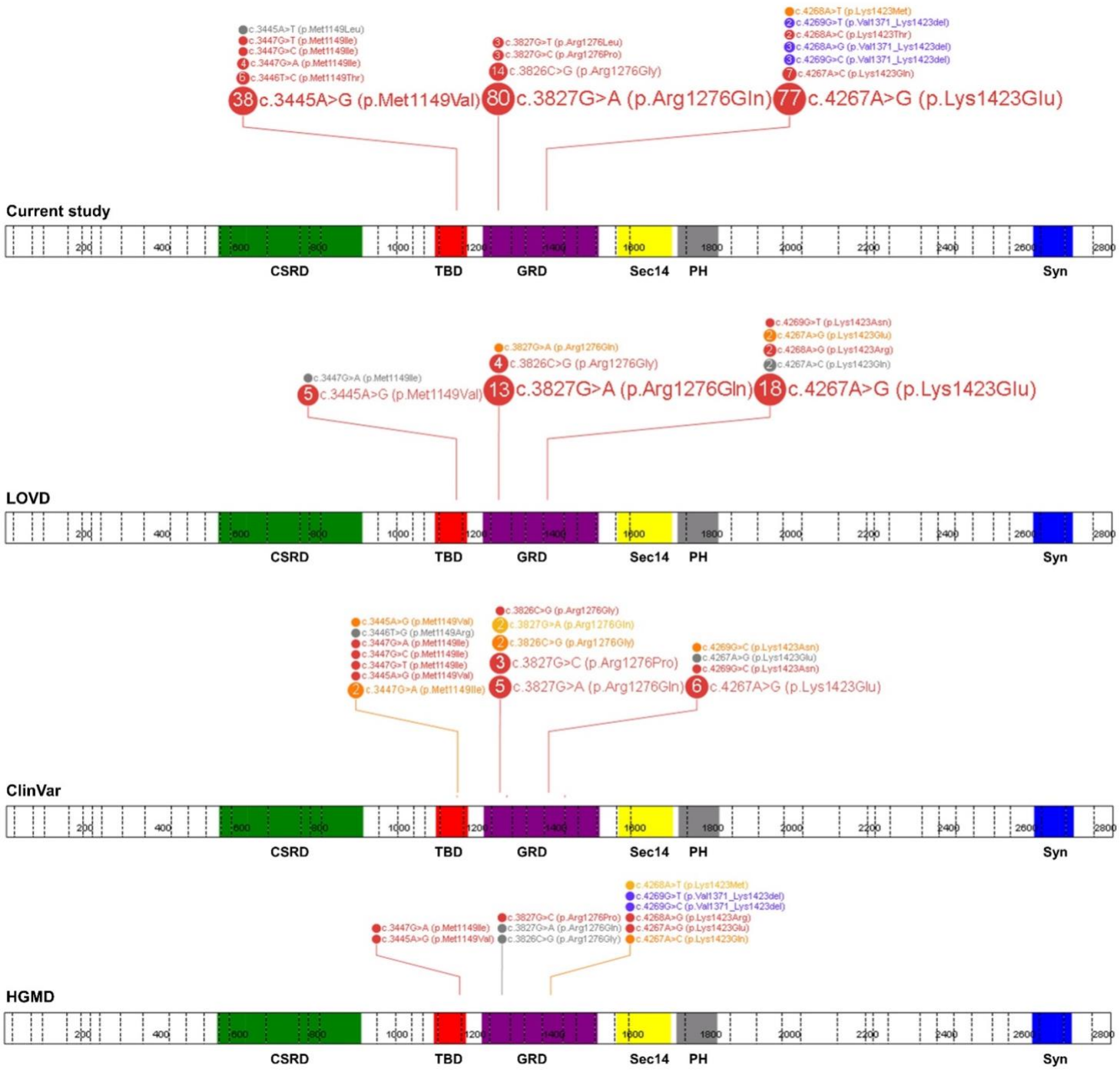
[A] According to Thissen et al. (2002); [B] No statistically significant *P*-value after B-H correction.

Supp. Table S31. The prevalence of pulmonic stenosis in the studied cohorts and in individuals heterozygous for pathogenic *NF1* missense variants affecting codons 1809 and 844-848, p.Met992del, the cohort of the individuals carrying one out of the 18 most frequent recurrent pathogenic nonsense variants observed in the University of Alabama at Birmingham (UAB) database and in the general NF1 population.

Study	N (%)	Reference
Pathogenic missense variants affecting codons 844-848	2/113 (1.8)	Koczkowska et al. (2018)
In-frame deletion p.Met992del	8/160 (5)	Upadhyaya et al. (2007); Koczkowska et al. (2019)
Pathogenic missense variants affecting p.Met1149	2/52 (3.9)	Current study
Pathogenic missense variants affecting p.Arg1276	11/92 (12)	Current study
Pathogenic missense variants affecting p.Lys1423	11/76 (14.5)	Current study
Pathogenic missense variants affecting p.Arg1809	14/132 (10.6)	Nyström et al. (2009); Ekvall et al. (2014); Pinna et al. (2015); Santoro et al. (2015); Rojnueangnit et al. (2015)
Pathogenic variants affecting codons 844-848, 992, 1149, 1276, 1423 and 1809	48/625 (7.7)	Upadhyaya et al. (2007); Nyström et al. (2009); Ekvall et al. (2014); Pinna et al. (2015); Santoro et al. (2015); Rojnueangnit et al. (2015); Koczkowska et al. (2018); Koczkowska et al. (2019); Current study
UAB nonsense variants cohort	13/701 (1.9)	Current study
General NF1 population	25/2,322 (1.1)	Lin et al. (2000)

Supp. Table S32. The overall prevalence of cardiac/cardiovascular abnormalities, excluding hypertension as the only feature, in the studied cohorts and in individuals heterozygous for pathogenic *NF1* missense variants affecting codons 1809 and 844-848, p.Met992del, the cohort of the individuals carrying one out of the 18 most frequent recurrent pathogenic nonsense variants observed in the University of Alabama at Birmingham (UAB) database and in the general NF1 population.

Study	N (%)	Reference
Pathogenic missense variants affecting codons 844-848	10/113 (8.9)	Koczkowska et al. (2018)
In-frame deletion p.Met992del	10/113 (8.9)	Koczkowska et al. (2019)
Pathogenic missense variants affecting p.Met1149	5/52 (9.6)	Current study
Pathogenic missense variants affecting p.Arg1276	19/92 (20.7)	Current study
Pathogenic missense variants affecting p.Lys1423	18/76 (23.7)	Current study
Pathogenic missense variants affecting p.Arg1809	16/105 (15.2)	Rojnueangnit et al. (2015)
UAB nonsense variants cohort	39/701 (5.6)	Current study
General NF1 population	54/2,322 (2.3)	Lin et al. (2000)

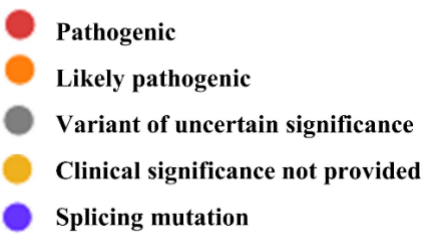


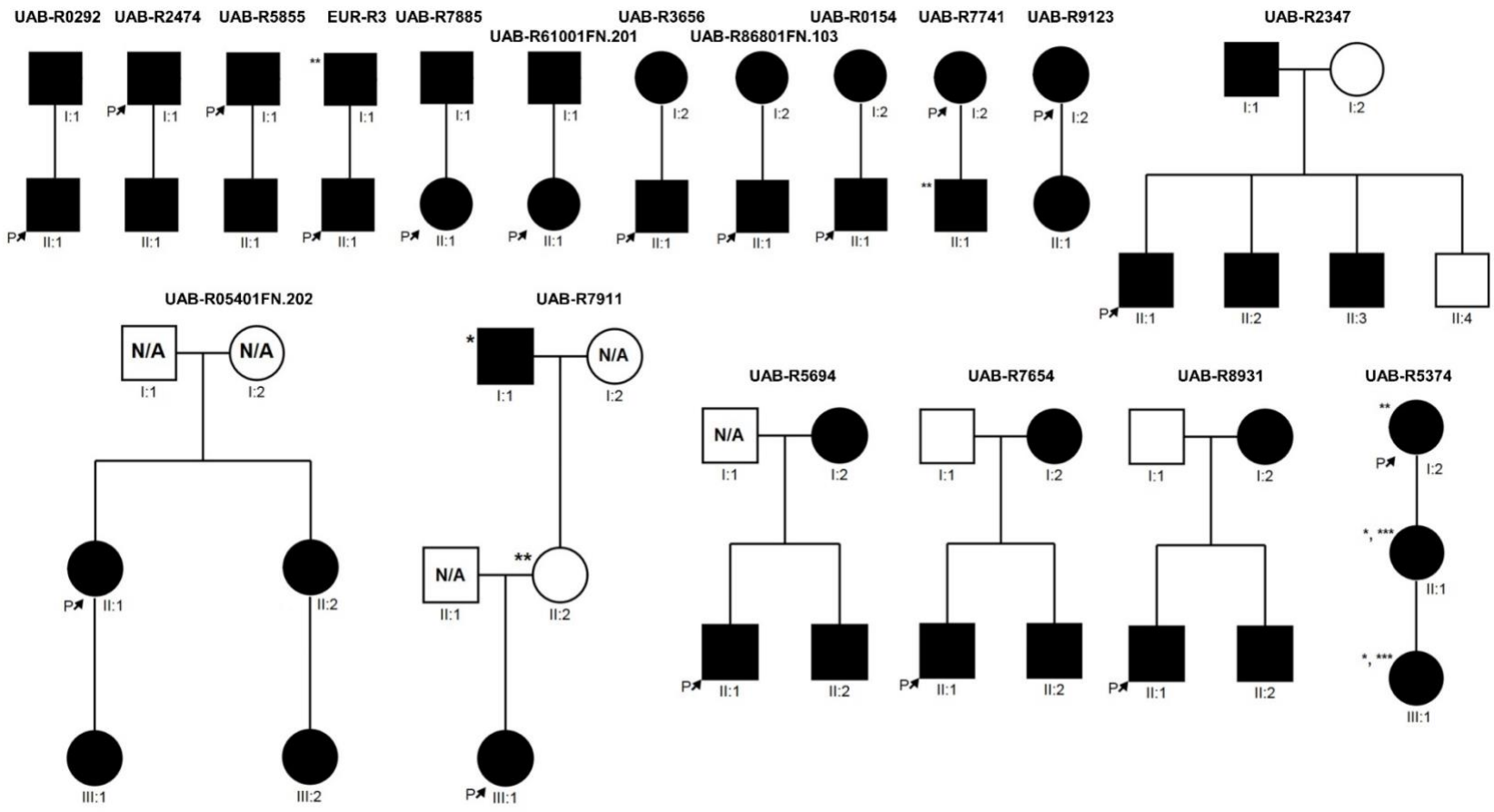
Supp. Figure S1. The classification of *NF1* missense variants affecting p.Met1149, p.Arg1276 and p.Lys1423: comparison of the classification reported in publicly available disease databases (HGMD, LOVD and ClinVar) with the current study (as of December 2018).

The classification of the variants deposited in the publicly available LOVD and ClinVar databases are shown here as reported by the original author(s). For variants deposited in the HGMD database, the originally reported variants' classifications were reviewed and if necessary re-classified according to the ACMG guidelines (Richards et al., 2015) based on the evidence for pathogenicity provided in the published papers by the authors (see details below). Each number in the circle corresponds to the total number of probands with a specific variant. Different colors correspond with the pathogenicity score according to the legend presented below (c.4268A>G, c.4269G>C and c.4269G>T are not *true* missense variants; these variants lead to skipping of exon 32 [24] during *NF1* mRNA splicing as observed by RNA-based analysis in blood). The figure was prepared using ProteinPaint application (Zhou et al., 2016).

HGMD: **c.3445A>G (p.Met1149Val)** and **c.4267A>C (p.Lys1423Gln)** absence in normal controls, segregation studies, proven *de novo* with paternity confirmation, *in silico* analysis (van Minkelen et al., 2014); **c.3447G>A (p.Met1149Ile)** - reported in 1/169 unrelated NF1 individuals, absent in normal controls and proven *de novo* (Griffiths et al., 2007); **c.3826C>G (p.Arg1276Gly)** - reported in 2/91 unrelated NF1 individuals fulfilling the NIH diagnostic criteria, no phenotypic details available, no evidence for pathogenicity provided (Mattocks et al., 2004); **c.3827G>A (p.Arg1276Gln)** - reported in 2/521 unrelated NF1 individuals, *in silico* analysis, no other evidence for pathogenicity provided (Fahsold et al., 2000); **c.3827G>C (p.Arg1276Pro)** - based on functional studies (Klose et al.,1998); **c.4267A>G (p.Lys1423Glu)** - segregation studies (variant reported in 5 NF1-affected family members) and functional studies (Li et al., 1992); **c.4268A>G (p.Lys1423Arg)** - reported once in sporadic case of 50 unrelated NF1 individuals, absent in normal controls, but no proven *de novo, in silico* analysis (Han et al., 2001); **c.4268A>T (p.Lys1423Met)** - no evidence for pathogenicity provided (Corsello et al., 2018); **c.4269G>C (p.Lys1423Asn)** - reported in 1/374 unrelated NF1 individuals, based on *NF1* screening with an RNA-based approach and *in-silico* analysis this variant was reported as skipping of exon 32 [24] during *NF1* mRNA splicing and to result in a low level of transcript with p.Lys1423Asn missense variant, r.[4269g>c,4111_4269del] (p.[Lys1423Asn,Val1371_Lys1423del]; Pros et al., 2008); **c.4269G>T (p.Lys1423Asn)** - absence in normal controls, disease phenotype segregated with the variant in familial cases, *in silico* analysis; however, no RNA-based approach, therefore missplicing was not recognized in the original paper (De Luca et al., 2005).

Abbreviations: **CSRD** - **c**ysteine-**s**erine **r**ich **d**omain; **TBD** - **t**ubulin **b**inding **d**omain; **GRD** - **G**AP (GTPase-activating protein) **r**elated **d**omain; **Sec14** - **Sec14** homology-like domain; **PH** - **p**leckstrin **h**omology-like domain; **Syn** - **syn**decan binding domain.

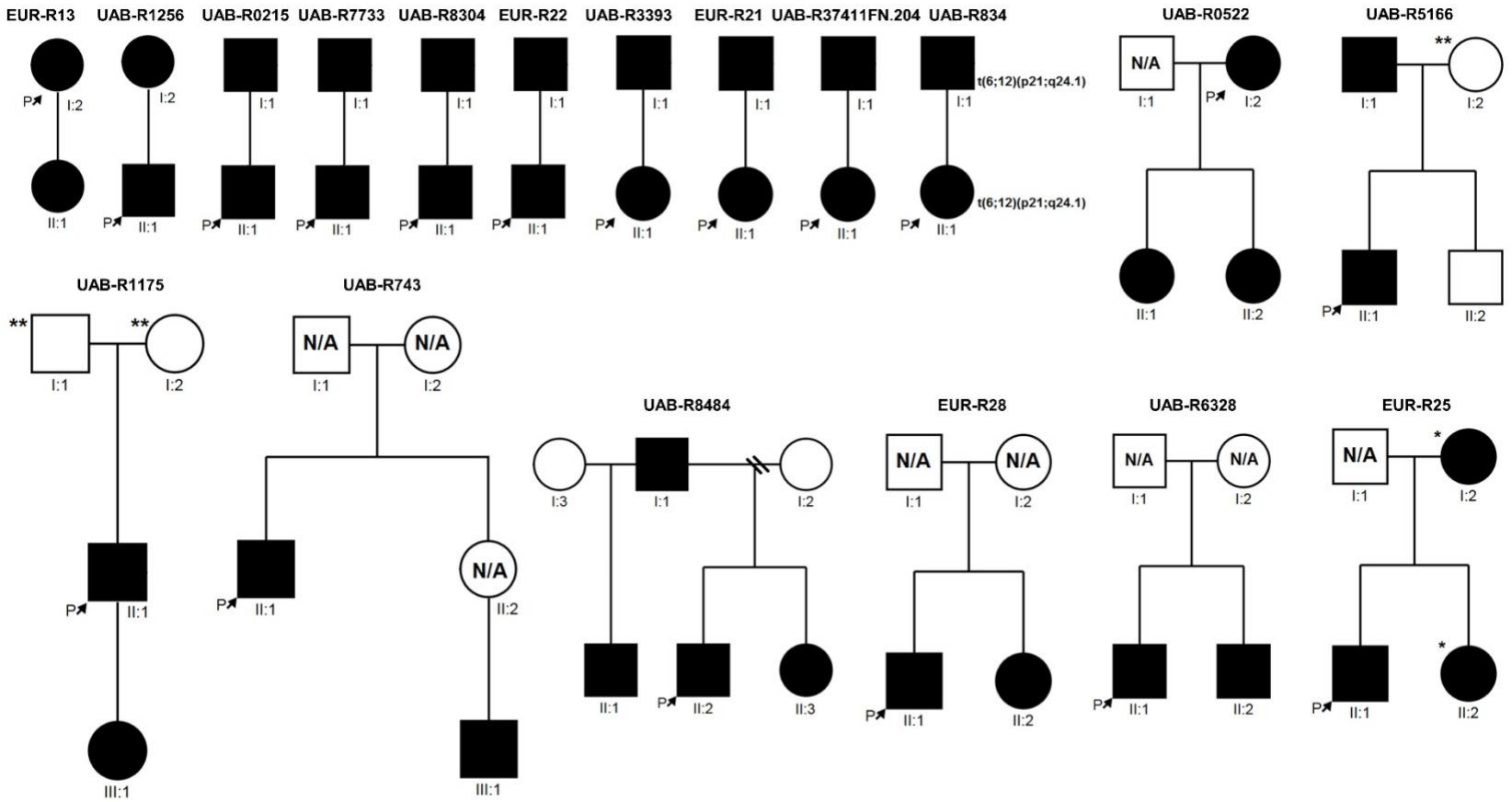




Supp. Figure S2. Pedigrees of the familial cases heterozygous for pathogenic *NF1* missense variants affecting p.Met1149 in the current study.

The filled black symbols represent the individuals who are positive for an *NF1* missense pathogenic variants affecting p.Met1149, while the open symbols represent relatives who are negative for the family specific variant. The arrow indicates the proband of the family. For the first generation, father is always I:1 and mother is I:2.

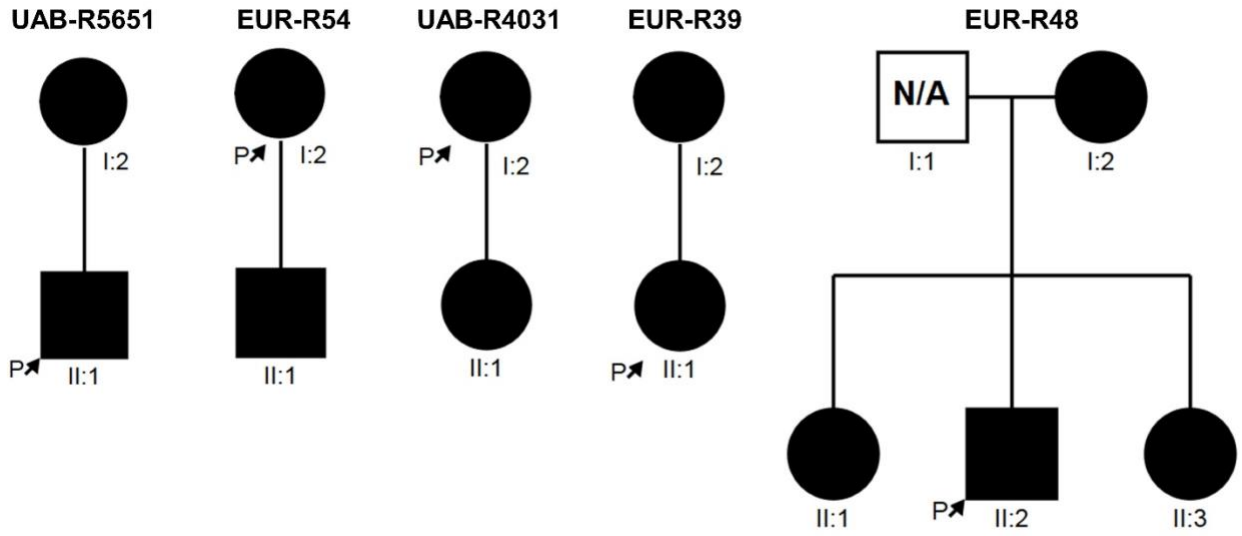
Abbreviations: **N/A** - the relatives not available for genetic analysis; ***** - no clinical information available for these individuals; ****** - no clinical signs of NF1, including no pigmentary manifestations reported in these individuals; ******* - *NF1* genetic testing not performed at UAB.



Supp. Figure S3. Pedigrees of the familial cases heterozygous for pathogenic *NF1* missense variants affecting p.Arg1276 in the current study.

The filled black symbols represent the individuals who are positive for an *NF1* missense pathogenic variants affecting p.Arg1276, while the open symbols represent relatives who are negative for the family specific variant. The arrow indicates the proband of the family. For the first generation, father is always I:1 and mother is I:2.

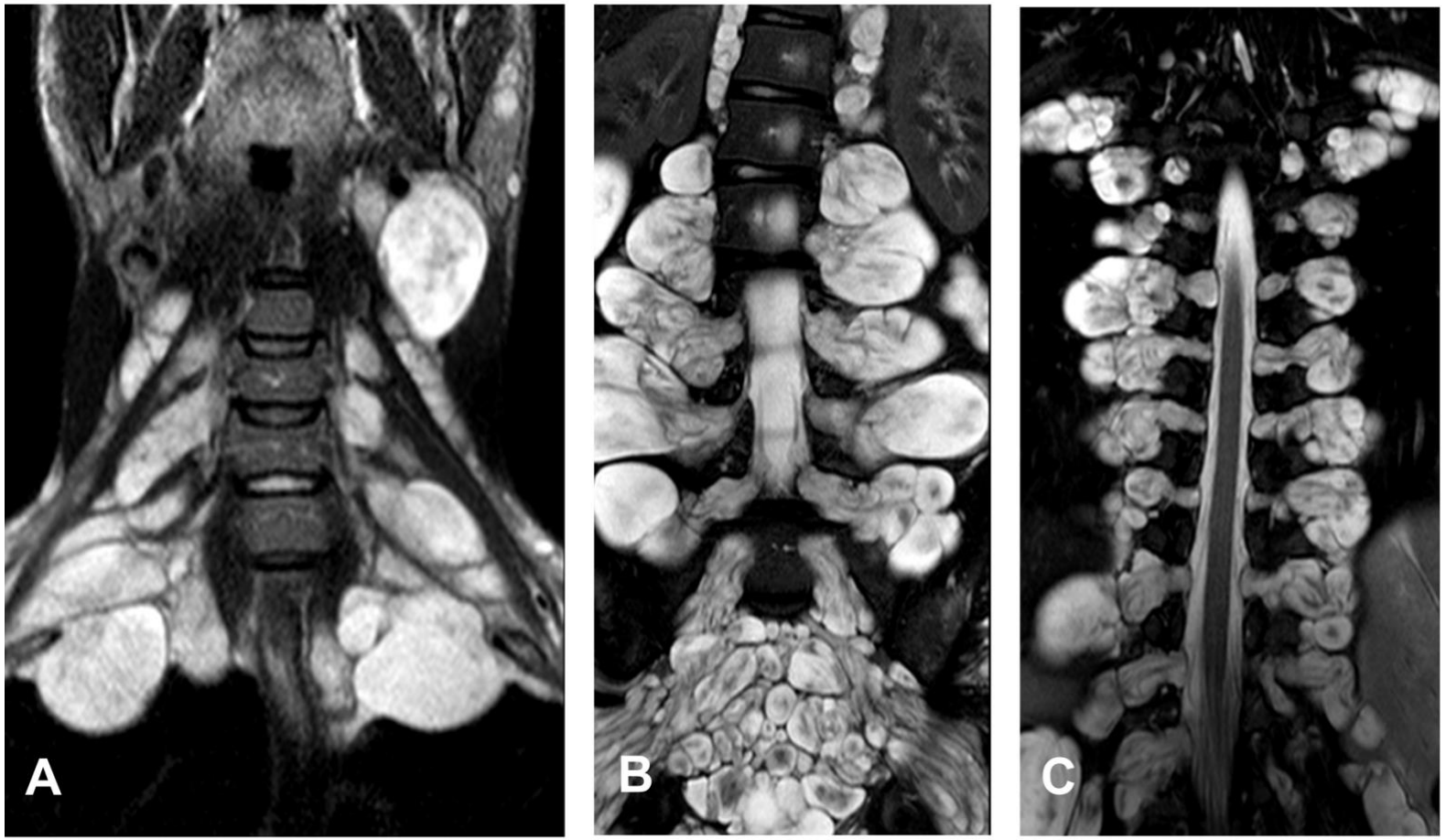
Abbreviations: **N/A** - the relatives not available for genetic analysis; ***** - no clinical information available for these individuals; ****** - no clinical signs of NF1, including no pigmentary manifestations reported in these individuals.



Supp. Figure S4. Pedigrees of the familial cases heterozygous for pathogenic *NF1* missense variants affecting p.Lys1423 in the current study.

The filled black symbols represent the individuals who are positive for an *NF1* missense pathogenic variants affecting p.Lys1423, while the open symbols represent relatives who are negative for the family specific variant. The arrow indicates the proband of the family. For the first generation, father is always I:1 and mother is I:2.

Abbreviations: **N/A** - the relatives not available for genetic analysis.



Supp. Figure S5. Coronal T2-weighted STIR MR images of the cervical (A), lumbosacral (B) and dorsal (C) spine showing bilateral giant and fairly confluent plexiform neurofibromas of all paraspinal nerves and plexuses in individual EUR-R22-F. Note the intraspinal tumor origin in all the spinal foramina which appear widening without extension in the spinal canal. Referred by Marica Eoli, MD (Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy).

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