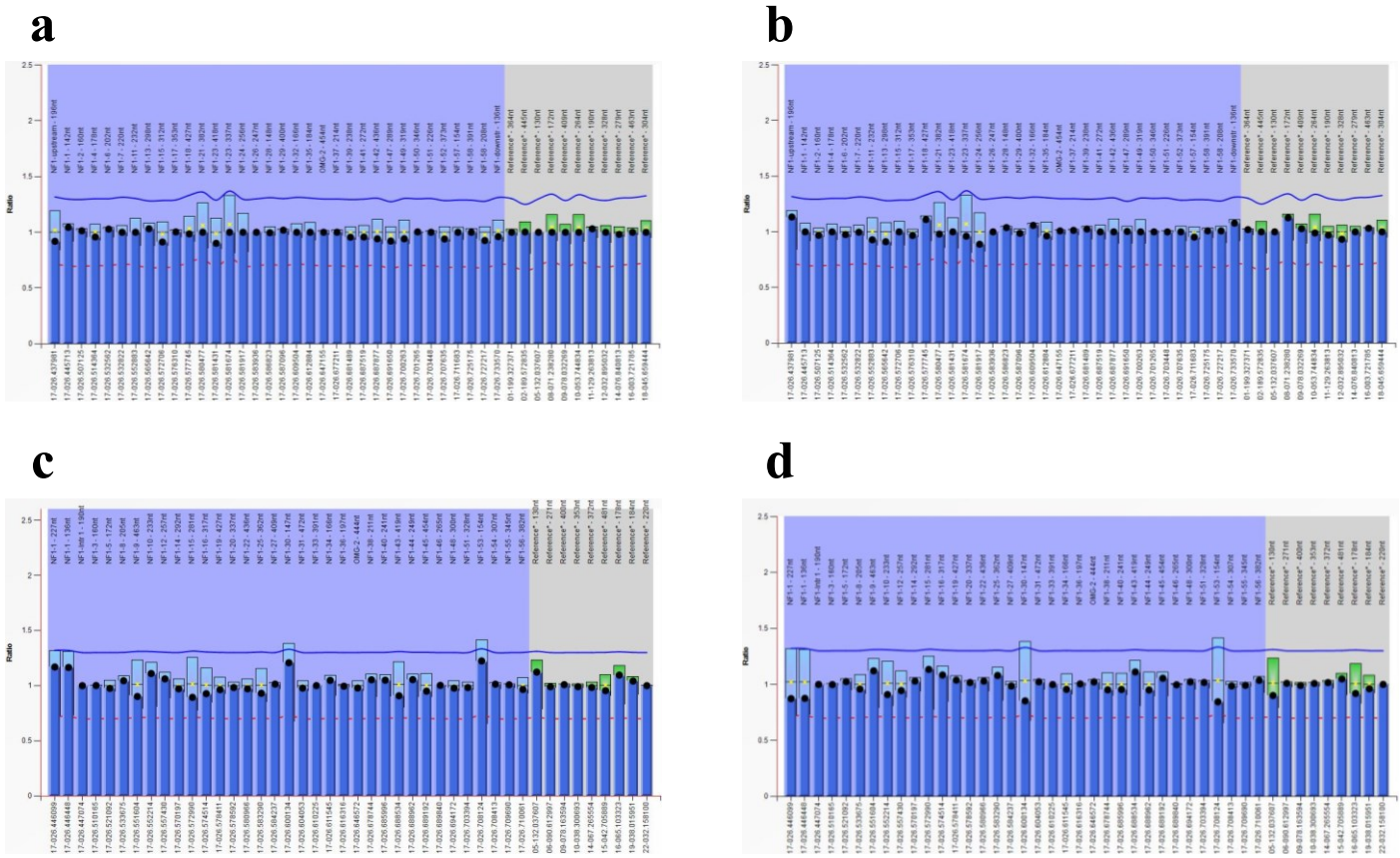


**Fig. S1** Pedigrees of families of sporadic cases. The arrows indicate the probands in each family. The asterisks denote that peripheral blood samples of individuals had been acquired. **a–e**: Pedigrees of Families 6–10



**Fig.S2** Multiplex ligation-dependent probe amplification (MLPA) results of P081 and P082 for patients in Family 12. **a**: MLPA results of P081 for Patient 22; **b**: MLPA results of P081 for Patient 23; **c**: MLPA results of P082 for Patient 22; **d**: MLPA results of P082 for Patient 23

<b>a</b>		629
		***** *
Human	KFLLKNKQADRSSCHFLFLFYGVGCDIPSSGNTSQMSMDHEE	
Chimpanzee	KFLLKNKQADRSSCHFLFLFYGVGCDIPSSGNTSQMSMDHEE	
Rhesus	KFLLKNKQADRSSCHFLFLFYGVGCDIPSSGNTSQMSMDHEE	
Bat	KFLLKNKQADRSS--FLFLYGGGCDVPSSGNTSQMSMDHEE	
Dog	KFLLKNKQADRSSCHFLFLFYGVGCDVPSSGTTSQMSMDHEE	
Rabbit	KFLLKNKQADRSSCHFLYLYGVGCDVPSSGNTSQMSMDHEE	
Mouse	KFLLKNKQADRSSCHSLYLYGVGCEMSATGNTTQMSVDHDE	
Goat	KFLLKNKQADRSSCHFLIVYGVGCDVPSSGNTSQMSMDHDE	
Horse	KFLLKNKQADRSSCHFLFLYGVGCDVPSSGNTSQMSMDHEE	
Chicken	KFLLKNKQSDRTSCHFLFLYDV-----SGGGASQMSLDHEE	
Toad	KFLLKYK-----	
Fish	KFLLKNK-----	

<b>b</b>		1490
		:*****:*****:*****
Human	AVNHSLSFISDGNVLALHRLWNNQEKIGQYLSNRDHKAV	
Chimpanzee	AVNHSLSFISDGNVLALHRLWNNQEKIGQYLSNRDHKAV	
Rhesus	AVNHSLSFISDGNVLALHRLWNNQEKIGQYLSNRDHKAV	
Bat	AVNHSLSFISDGNVLALHRLWNNQEKIGQYLSNRDHKAV	
Dog	TVNHSLSFISDGNVLALHRLWNNQEKIGQYLSNRDHKAV	
Rabbit	AVNHSLSFISDGNVLALHRLWNNQEKIGQYLSNRDHKAV	
Mouse	AVNHSLSFISDGNVLALHRLWNNQEKIGQYLSNRDHKAV	
Goat	AVNHSLSFISDGNVLALHRLWNNQEKIGQYLSNRDHKAV	
Horse	AVNHSLSFISDGNVLALHRLWNNQEKIGQYLSNRDHKAV	
Chicken	TVNHSLSFISDGNVLALHRLWNNQEKIGQYLSNRDHKAV	
Toad	TVNHSLSFITDGNVLALHRLWNNQEKIGQYLSNRDHKAV	
Fish	SVNHSLSFISDGNVLALHRLWNNQERIGQYLSNRDHKAV	

<b>c</b>		1931
		***** ***** .*****:
Human	SGFSKSSIELKHLCEYMPWLSNLVRFCKHNDDAKRQRT	
Chimpanzee	SGFSKSSIELKHLCEYMPWLSNLVRFCKHNDDAKRQRT	
Rhesus	SGFSKSSIELKHLCEYMPWLSNLVRFCKHNDDAKRQRT	
Bat	SGFSKSSIELKHLCEYMPWLSNLVRFCKHNDDAKRQRT	
Dog	SGFSKSSIELKHLCEYMPWLSNLVRFCKHNDDAKRQRT	
Rabbit	SGFSKSSIELKHLCEYMPWLSNLVRFCKHNDDAKRQRT	
Mouse	SGFSKSSIELKHLCEYMPWLSNLVRFCKHNDDAKRQRT	
Goat	SGFSKSSIELKHLCEYMPWLSNLVRFCKHNDDAKRQRT	
Horse	SGFSKSSIELKHLCEYMPWLSNLVRFCKHNDDAKRQRT	
Chicken	SGFSKSSIELKHLCEYMPWLSNLVRFCKHNDDAKRQRT	
Toad	SGFSKSSIELKHLCEYMPWLLNLVRFCKLTDDAKRQRT	
Fish	SGFSKSSIELKHLCEYMPWLLNLVRFCKHNDDAKRQRT	

**Fig. S3** Amino acid sequences of neurofibromin around missense mutations. Mutation sites are highlighted. **a:** The amino acid G629 and surrounding sequence; **b:** The amino acid L1490 and surrounding sequence; **c:** The amino acid W1931 and surrounding sequence

**Table S1** Primers used in this study

Application	Amplicon	Sequence (5'-3')	T <sub>m</sub> (°C)	Size (bp)
Sanger Sequencing	<i>NFI-E9-11</i>	F: GAGTTTTAGAGGCTGTAAATTTGC	55	2076
		R: GGCAAATGAGGTGAAGGGTT	60	
	<i>NFI-E16</i>	F: GTCTCTGCTGTGCATGTGGTTTATT	59	752
		R: ACAATATGCTCCTCAAGCTACTCTG	59	
	<i>NFI-E17</i>	F: TAATGAATTCATAGTGCCCTGGTAG	57	969
		R: TGGGTTTTTTATGGAGTGGAG	54	
	<i>NFI-E18</i>	F: AGTTCTTGTTTATGCCCTTGTTTC	55	776
		R: TGTGCTTTGAGGCAGACTGAGTA	58	
	<i>NFI-E28-29</i>	F: CTTTGGGTTTACATTTTTGCTACTC	56	897
		R: ATTACCTATTAGCCTCTTCCCTTC	57	
	<i>NFI-E33-34</i>	F: GCATAGGATTCATATTCTGGCACT	57	2328
		R: CATTAACCTTTATGAGGGTTCTG	55	
	<i>NFI-E36</i>	F: CAGGGTTCCAGTTTGTCTATTTTC	57	1528
		R: CATATCCTCCACAGGACCTCCAT	60	
	<i>NFI-E37</i>	F: TTAGGCTGGAAGTAGAAGAGTGT	57	1521
		R: GAGTGATGTAGGTGGAAAGTATG	57	
<i>NFI-E39</i>	F: GTTCTTTCTTCGCCTCTACAA	54	439	
	R: GCTTTGCCCAATGTGGCACC	60		
<i>NFI-E40-41</i>	F: TTCATATTGATTAGGCTGTTCC	53	919	
	R: CACTGAGTATTCCGCTTATGG	56		
<i>NFI-E45</i>	F: TCCGAGATTCAGTTTAGGAGTT	54	580	
	R: ATCCATTCACTCCCCAACCT	55		
Mutation Validation	<i>NFI-Family 1</i>	F: TTCTCATAGAAATAATCTGCTT	49	131
		R: CCTTTTGGACTIONACATTGGT	56	
	<i>NFI-Family 2</i>	F: ACTGGTCAAATCAATGGTGATA	53	101
		R: GTATGGGTAAGGTCCACTACAA	56	
	<i>NFI-Family 3</i>	F: AAGCCATTGTCCAGTCTATCA	54	151
		R: CTCGGGTCAGAACTGCCTAA	62	

Abbreviation: T<sub>m</sub>: melting temperature calculated by Primer Premier 5 of the primer; E: exon; F: forward; R: reverse.

**Table S2** *In silico* analysis of missense mutations

Mutation Position		Exon 17	Exon 33	Exon 39
Nucleotide Change		c.1885G>A	c.4469T>C	c.5791T>C
Amino Acid Change		p.G629R	p.L1490P	p.W1931R
UCSC	dbSNP 147	rs199474738	-	rs199474791
Genome	Allele Frequencies	A: 0.002% (2 / 121202);		-
Browser		G: 99.998% (121200 / 121202)		
ExAC	Variant	17:29552152 G/A		
	Allele Count	2		
	Allele Number	121202		
	Number of Homozygotes	0		
	Allele Frequency	0.0000165		
Prediction	PolyPhen-2	Probably Damaging	Probably Damaging	Probably Damaging
Tool	SIFT	Tolerated	Affect Protein Function	Affect Protein Function
	Mutation Taster	Disease Causing	Disease Causing	Disease Causing