The genetic spectrum of *NF1* variants in 10 unrelated Chinese families with neurofibromatosis type 1

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ABSTRACT

الأهداف: دراسة المظاهر السريرية والوراثية في مجموعة من العائلات الصينية المصابة بالورم الليفي العصبي من النوع1 (NF1).

المنهجية: أجرينا تحليل المعلومات السريرية لـ 21 مريضا يعانون من NF1 في 10 عائلات بأثر رجعي. لتوسيع الطيف الوراثي لـ NF1، وأجرينا تحليل تضخيم مسبار يعتمد على الارتباط المتعدد أولاً، يليه تسلسل الإكسوم الكامل، من أجل تحديد المتغيرات المسببة للأمراض أو المسببة للأمراض لجين *NF1* في 10 عائلات صينية غير مرتبطة.

النتائج: تم تحديد تسعة أنواع مختلفة من *NF1* في جميع العائلات العشر. من بين هذه، 7 كانت متغيرات مسببة للأمراض معروفة وتضمنت حذف اكسون 1، وحذف اكسون 58-1، c.5401C>T (p.Q1801*)، c.2291-2A>C، c.484C>T (p.Q162*)، c.4922G>A (p.W1641*)، c.1019_1020del (p.S340Cfs*25)). المتغيران الجديدان هما c.5197T>C (p.S1733P) و(c.783_797delinsC (p.K261Nfs*25). تم تصنيف متغير p.S1733P على أنه متغير ذو أهمية غير مؤكدة، في حين تم تصنيف p.K261Nfs*25 على أنه مسبب للأمراض. وبعد ذلك، كان معدل الاكتشاف الإيجابي لمتغيرات *NF1* 100% (10/10)). بينما كانت المتغيرات المقتطعة مسؤولة عن 60.0% (6/10) من الحالات، كان متغير الربط مسؤولاً عن 10% (1/10) من الحالات.

الخلاصة: لقد حددنا متغيرين جديدين متغايري الزيجوت c.5197T>C و c.783_797delinsC في جين *NF1*، مما يوسع الطيف الوراثي لجين *NF1*.

Objectives: To investigate the clinical and genetic features in a cohort of Chinese families with neurofibromatosis type 1 (NF1).

Methods: The clinical information of 21 patients with *NF1* in 10 families was retrospectively analyzed. To broaden the genetic spectrum of *NF1*, multiplex ligation-dependent probe amplification analysis was performed first, followed by the whole-exome sequencing, in order to identify pathogenic or potentially pathogenic variants of *NF1* gene in 10 unrelated Chinese families..

Results: Nine different *NF1* variants were identified in all 10 families. Of these, 7 were known pathogenic variants and included the exon 1 deletion, exons 1-58 deletion, c.5401C>T (p.Q1801*), c.2291-2A>C, c.484C>T (p.Q162*), c.4922G>A (p.W1641*) and c.1019_1020del (p.S340Cfs*25). The 2 novel variants were c.5197T>C (p.S1733P) and c.783_797delinsC (p.K261Nfs*25). The p.S1733P variant was classified as a variant of uncertain significance, while p.K261Nfs*25 was classified as pathogenic. Hence, the positive detection rate of *NF1* variants was 100% (10/10). While the truncating variants were responsible for 60.0% (6/10) of the cases, the splicing variant was responsible for 10% (1/10) of the cases.

Conclusion: We identified 2 novel heterozygous variants (c.5197T>C and c.783_797delinsC) in the *NF1* gene, which broadens the genetic spectrum of the *NF1* gene.

Neurosciences 2024; Vol. 29 (3): 177-183
doi: 10.17712/nsj.2024.3.20230003

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Received 8th January 2024. Accepted 31th May 2024.

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Disclosure. The authors declare no conflicting interests, support or funding from any drug company.

Neurofibromatosis type 1 (*NF1*, OMIM: 613113) is an autosomal dominant genetic diseases, with an estimated incidence of about 1 in 2500-5000 individuals.[1] The typical clinical manifestations are skin changes including multiple café-au-lait macules (CALMs), freckling, lisch nodules (iris hamartomas), and peripheral neurofibromas.[2] Previous studies have reported that the symptoms are age-dependent. While nearly half of the children with *NF1* showed symptoms before the age of one, all the children were symptomatic by the time they turned 20 years.[3] Furthermore, *NF1* patients are prone to benign or malignant tumors, skeletal anomalies, and cognitive impairments, which result in psychological burden and nursing problems. Although *NF1* is a monogenic disorder, there is high variability in the disease outcomes. The clinical symptoms can vary within a family or even at different life stages of the same patient.[4-5] Therapeutic interventions include genetic counseling, surgical resection of neurofibromas and treatment with mitogen-activated protein kinase (MEK) inhibitors.[5]

The disease-causing gene *NF1*, with a 350kb genomic size and 58 exons, is located on chromosome 17q11.2 and has 3 alternatively spliced exons. *NF1* is ubiquitously expressed and encodes neurofibromin, a highly conserved Ras GTPase-activating protein (GAP). It down regulates Ras/MAPK/AP-1 by converting active GTP into inactive GDP.[6-7] Due to the high incidence of variants, *NF1* gene has a high rate of spontaneous mutations. At least 3800 pathogenic variants have been identified till now, as per the HGMD database, (http://www.hgmd.cf.ac.uk/ac/index.php, updated on Dec 2023). The *NF1* gene variants include insertions, stop and splicing mutations, whole gene deletions, amino acid changes and chromosome rearrangements. Approximately 50% of *NF1* cases are due to de novo variants and lack positive family history. The de novo variants occur primarily in paternally derived chromosomes. The percentage of single nucleotide substitutions and small deletions, which include nonsense, missense or splicing mutations, was 85-90%. Moreover, large deletions or entire *NF1* gene could be seen in 1-5% of the people having variants of the *NF1* gene, who often had a higher incidence of cognitive impairment and dysmorphic facial features, and an earlier appearance of cutaneous neurofibromas.[8] Thus, molecular testing could be helpful to identify the pathogenic variant in nearly 95 percent of *NF1* patients.[9] A negative genetic test might be caused by mosaicism or may represent a different disorder. Although a genotype-phenotype correlation has been observed in some *NF1* cohorts, there is considerable genetic heterogeneity, inter- and intra-familial variability and dependency of phenotype on age. Further research is needed to elucidate the roles of the various causative factors in their entirety.[10]

In the present study, we recruited 10 unrelated Chinese patients who had been clinically diagnosed with *NF1*. We performed genetic investigation in these cases using multiplex ligation-dependent probe amplification (MLPA), in combination with whole-exome sequencing (WES), followed by verification using Sanger sequencing. We found 9 different *NF1* variants in all 10 index patients, including 7 reported variants and 2 novel variants. The present study broadens the *NF1* variants spectrum in *NF1* patients from southeastern China.

Methods. From December 2020 to March 2023, 10 index patients (8 familial cases and 2 sporadic cases) with *NF1* were recruited for this study. The patients' information was collected and met the diagnostic criteria of *NF1*.[1] Our study was approved by the Ethics Committee. Written informed consent was obtained from the participants or their legal guardians.

Genomic DNA was extracted using standard methods. Large deletions or duplications in *NF1* and *NF2* was examined using MLPA kits (MRC-Holland, the Netherlands), according to the manufacturer's recommendations.[11-12]

The WES was performed using the methods reported previously. SIFT (https://sift.ssec.wisc.edu/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) softwares were used to predict the functional changes in proteins. The pathogenicity of the identified variants was further classified based on the criteria laid down by the American College of Medical Genetics and Genomics (ACMG). All variants were further validated by Sanger sequencing. The primer sequences for Family 2 were 5'-CTTCTCCACTTCACCCCGTC-3' and 5'-ACTCGGGTCAGAACTGCCTA-3'; Family 4: 5'-GGCCATTCTTTACTGCACACAAA-3' and 5'-TCCTCCTTTCTACCAATAACCGCA-3'; Family 5: 5'-CTATAGGTGTGTGCCATCATG-3' and 5'-GACCCAGTGATTTTTTTCAG-3'; Family 6: 5'-CTATAACTGTAACTCCTGGGTC-3' and 5'-CCCGGCTAATGTTTGTATTT-3'; Family 7: 5'-CTTAGCCTTATTTCTCAGTGTCCA-3' and 5'-GCCTTTTGCTACCTTTGAGGC-3'; Family

Table 1 - Clinical data of the 21 NF1 patients in 10 families.

Pedigree number	Family history	Patient number	Individual	Age at diagnosis (year)	Age at onset (year)	Gender	>5 CALMs
1	Yes	1	III:4	20	3	Female	Yes
2	No	2	II:2	16	Infancy	Female	Yes
3	Yes	3	III:2	12	8	Female	Yes
		4	II:1	42	6	Male	Yes
		5	III:1	14	6	Male	Yes
		6	I:1	66	10	Male	Yes
4	Yes	7	II:1	56	Infancy	Male	Yes
		8	III:2	28	Infancy	Female	Yes
5	Yes	9	II:2	55	18	Male	Yes
		10	III:1	16	10	Male	Yes
6	Yes	11	III:1	25	Infancy	Female	Yes
		12	II:4	49	Infancy	Male	Yes
7	Yes	13	III:1	52	2	Female	Yes
		14	II:1	76	2	Male	Yes
		15	II:2	72	2	Female	Yes
		16	IV:2	30	2	Male	Yes
8	Yes	17	I:2	40	Infancy	Female	Yes
		18	II:1	9	Infancy	Female	Yes
9	Yes	19	II:1	33	16	Male	Yes
		20	I:1	62	10	Male	Yes
10	No	21	II:1	43	Infancy	Female	Yes

CALMs, café-au-lait macules; NA, not available; -, none.

Table 1 - Clinical data of the 21 NF1 patients in 10 families.

Pedigree number	Skinfold freckling	Cutaneous Neurofibromas	Plexiformneurofibromas	Lisch nodules	Optic glioma	Osseous lesions	Other tumors or cardiovascular defects	Intellectual disability
1	NA	-	-	-	-	-	-	-
2	NA	-	-	-	-	-	-	-
3	NA	-	-	-	-	-	Glioma	-
	Yes	Yes	-	-	-	-	-	-
	NA	-	-	-	-	-	-	-
	Yes	Yes	-	-	-	-	-	-
4	-	Yes	-	-	-	-	-	-
	-	Yes	-	-	-	-	-	-
5	Yes	Yes	-	-	-	-	-	-
	-	Yes	-	-	-	-	-	-
6	-	Yes	-	-	-	-	-	-
	Yes	Yes	-	-	-	-	-	-
7	-	Yes	-	-	-	-	-	-
	-	Yes	-	-	-	-	-	-
	-	Yes	-	-	-	-	-	-
	-	Yes	-	-	-	-	-	-
8	-	Yes	-	-	-	-	-	-
	-	Yes	-	-	-	-	Intracranial aneurysm	Cognition impairment
9	-	Yes	-	-	-	-	-	-
	-	Yes	-	-	-	-	-	-
10	-	Yes	-	-	-	-	Epilepsy	-

CALMs, café-au-lait macules; NA, not available; -, none

8: 5'-GAAGCTGTTCAGTCTTTGTTGCT-3' and 5'-TGCCCTCCTTACCTTATTCATGT-3'; Family 10: 5'-CTGGACAGTCTACGAAAAGCTCT-3' and 5'-AGTAAAATCCAGCTGCCAGAAGA-3'. Cosegregation analyses were performed on all available family members.

Results. The demographic features of the 10 families, including 21 patients, are shown in **Table 1** and genograms are shown in **Figure 1**. All the patients showed symptoms of the disorder before the age of 20; more than half of them presented skin changes before the age of 2 years. Multiple CALMs were observed in

Table 2 - The identified variants in the present study.

Family Number	Ref seq NM	Nucleotide change	Amino Acid Change	Variant Type	SIFT	Polyphen2	ACMG
1	NM_000267.3	Exons 1-58 deletion	/	Deletion	D	D	pathogenic
2	NM_000267.3	c.5401C>T	p.Q1801*	Nonsense	D	D	Likely pathogenic
3	NM_000267.3	Exon 1 deletion	/	Deletion	D	D	pathogenic
4	NM_000267.3	c.2991-2A>C	/	Splicing	D	D	Likely pathogenic
5	NM_000267.3	c.484C>T	p.Q162*	Nonsense	D	D	pathogenic
6	NM_000267.3	c.5197T>C	p.S1733P#	Missense	T	D	Uncertain significance
7	NM_000267.3	c.4922G>A	p.W1641*	Nonsense	D	D	pathogenic
8	NM_000267.3	c.783_797delinsC	p.K261Nfs*25#	Frameshift	D	D	Likely pathogenic
9	NM_000267.3	Exons 1-58 deletion	/	Deletion	D	D	pathogenic
10	NM_000267.3	c.1019_1020del	p.S340Cfs*25	Frameshift	D	D	pathogenic

D (SIFT) possibly damaging; T (SIFT), tolerated; D (Polyphen2), probably damaging; #, novel variants.

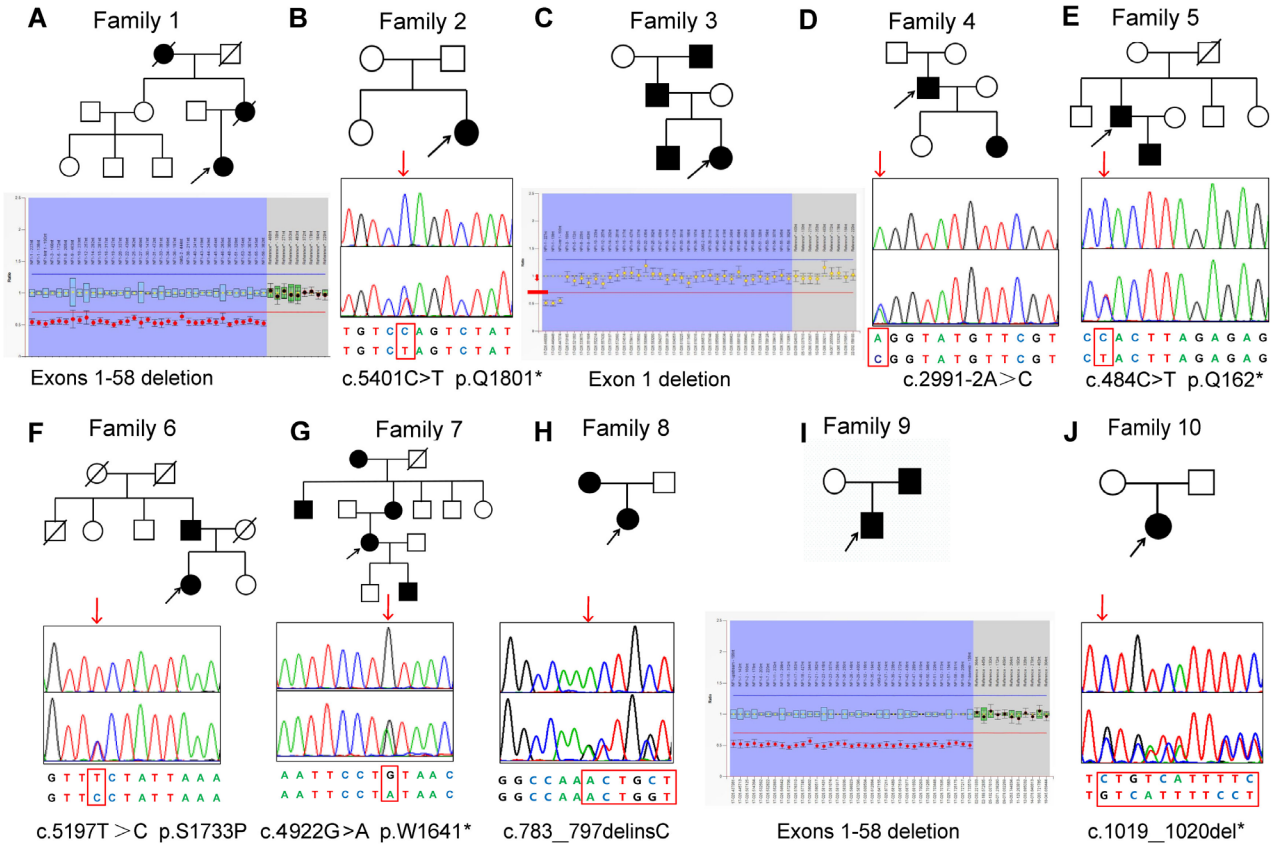

Figure 1 - Ten pedigree charts of families with *NF1*. The black symbols represent the affected individuals, and the open symbols represent the unaffected individuals. The circles and squares indicate females and males, respectively. The arrows identify the probands in the families. The second line represents the sequencing chromatograms of the *NF1* variants. The upper panel in the chromatogram depicts the reference sequence. The lower panel represents the heterozygous mutated sequence.

all 21 patients, and were found immediately after birth in patients 2, 7, 8, 11, 12, 17, 18, and 21. Seventeen patients suffered from cutaneous neurofibromas, and patients 4, 6, 9 and 12 had axillary or inguinal freckling. Skin lesions could be seen on the trunk in all 21 (100%) patients. Thirteen (61.9%) patients had these lesions on their limbs, while 3 (14.3%) patients had them on their necks and 5 (23.8%) on their faces. No plexiform

Homo sapiens (Human)	ALKLAHKDTKVSIKVGSTAVQVTS
Mus musculus (Mouse)	ALKLAHKDTKVSIKVGSTAVQVTS
Rattus norvegicus (Rat)	ALKLAHKDTKVSIKVGSTAVQVTS
Ovis aries (Sheep)	ALKLAHKDTKVSIKVGSTAVQVTS
Gallus gallus (Chicken)	ALKLAHKDTKVSIKVGSTAVQVTS
Canis lupus familiaris (dog)	ALKLAHRDTKVSIKVGSTAVQVTS

Figure 2 - Sequence alignment of the p.Ser1733 residue (Red letters) of *NF1* in different species.

neurofibroma was observed in any of the 21 *NF1* patients. Additionally, a glioma was found in patient 3, while patients 18 and 21 had intracranial aneurysm and epilepsy, respectively. Eighty percent (8/10) of the *NF1* pedigrees were consistent with autosomal dominant pattern; the 2 with a de novo variant were the only exceptions.

The MLPA analysis revealed that the 3 unrelated *NF1* patient probands carried an exon deletion in *NF1*. One patient had a single heterozygous exon 1 deletion and 2 patients had exon 1-58 deletions (**Table 2**). The other 7 patients with negative MLPA results proceeded to the WES analysis.

The WES was carried out in 7 *NF1* probands. After filtering, 5 known pathogenic nonsense variants (NF1: c.5401C>T, p.Q1801*; c.2991-2A>C; c.484C>T, p.Q162*; c.4922G>A, p.W1641*; and c.1019_1020del, p.S340Cfs*25), and 2 novel variants (NF1: c.5197T>C, p.S1733P; c.783_797delinsC, p.K261Nfs*25) were identified in 7 NF1 patients (**Table 2**). All the variants were confirmed by Sanger sequencing. The novel variant was absent in GnomAD and 1000G, as well as the 500 normal controls. Based on the ACMG standards, missense variant c.5197T>C (p.S1733P) was classified as a variant of uncertain significance, while the nonsense variant c.783_797delinsC was classified as pathogenic. Multiple sequence alignment analysis showed that the residue p.Ser1733 is conserved in different species (**Figure 2**). All the identified pathogenic variants are summarized in **Table 2**.

The novel *NF1* variant c.5197T>C (p.S1733P) (**Figure 1F**) was identified in a 25-year-old female who was born of a non-consanguineous marriage. Since birth, the girl had axillary and inguinal freckling, along with CALMs. She developed a cutaneous nodule on her back at the age of 8, with additional nodules gradually forming over time. The cutaneous nodules varied in size, ranging from 0.2 cm to 2.0 cm in diameter. She was diagnosed with NF1 after undergoing a nodule biopsy. Finally, the segregation analysis showed that her father carried this variant and also had CALMs and freckling.

The second novel *NF1* variant c.783_797delC (p.K261Nfs*25) (**Figure 1H**) was found in a 9-year-old girl from Zhejiang province. She presented with multiple CALMs scattered on her skin at birth. Her mother found a cutaneous nodule on her trunk at the age of 5, with the number gradually increasing with age. She had a spontaneous cerebral hemorrhage when she was 9-years-old. The post-operative pathology indicated cerebral aneurysm. The co-segregation analysis indicated that her mother carried the same variant, in addition to having CALMs and freckling when she was born.

Discussion. In our current study, MLPA and WES were performed in 10 unrelated clinically diagnosed *NF1* patients from southeast China. All patients were genetically diagnosed with *NF1*. Nine *NF1* gene variants (2 of them were novel variants) were present in all 10 unrelated families. Among the variants were four nonsense variants (p.Gln1801*, p.Gln162*, p.Trp1641*, and p.S340Cfs*25), one splicing variant c.2991-2A>C, one single exon 1 deletion, one multi-exon deletion (exons 1-58 deletion), and 2 novel variants (p.S1733P and p.K261Nfs*25). The variants included nonsense, frameshift, splicing, missense and deletion variants—90% of which could result in a truncated protein and the loss of protein function. In our cohort, the detection rate of positive variants was 100% (10/10).

A truncated neurofibromin may degrade and lead to the inactivation of the protein.[13] We detected 6 different truncating variants (3 nonsense, 2 frameshift, and one splicing variants), which represented 60.0% of all the variants identified. Furthermore, we detected 3 large deletion variants with a prevalence of 30.0%. The missense variant was less frequent, with a prevalence of 10.0%. Two of the detected variants (20.0%) occurred as de novo variants in the *NF1* patients, which was lower than usually reported frequencies.[14] Therefore, the *NF1* pathogenic variants were detected in 100% (10/10) of *NF1* patients, which is in line with the results of the previous studies performed in the populations from Southern Italy (96.0%, 70 of 73)[15] and Spain (97.5%, 78 of 80).[16] Splicing variants in NF1 account for 22-30% of *NF1* patients with positive family history. Most *NF1* pathogenic splicing variants cause the *NF1* pathology via a haploinsufficiency mechanism, which leads to the creation of novel splice sites or the activation of cryptic splice sites within exonic and intronic sequences. This results in frameshifts and premature stop codons.[17,18] Our study identified a novel *NF1* splicing variant at the splice donor site of intron 22 of the *NF1* gene. Furthermore, there are 6 previously reported pathogenic variants (c.2291-11T>G, c.2291-2A>G, c.2291-2A>T,

c.2291-1G>A, c.2291-1G>C and c.2291-1G>T) adjacent to this region. According to the HGMD, 3872 *NF1* variants have been reported worldwide to date, of which 507 are splicing variants. This suggests that splicing variants should not be ignored in *NF1* patients.

A few studies from China had reported the presence of *NF1* variants.[5,19-23] However, no obvious correlation between the genotype and clinical features was reported in these studies.[19,22] In a recent study, Zhu et al compared the clinical phenotypes with the different domains of neurofibromin in 2024. They deduced a few novel genotype-phenotype correlations in a large cohort of Chinese NF1 patients. For example, protein kinase C domain (PKC) disruption is associated with a higher rate of cutaneous neurofibromas. The patients with variations in the cysteine-serine rich domain showed a higher rate of second primary malignancy.[10] However, caution should be exercised while drawing conclusions in other cohorts of *NF1* patients.

Neurofibromin contains a RAS-GTPase-activating domain, which converts the active p21-RAS-GTP to the inactive p21-RAS-GDP.[6] Neurofibromin regulates cell proliferation and differentiation as a RAS-MAPK signal.[6,24] More than 3800 different variants have been identified in the *NF1* gene until now. However, no hot-spot variants have been reported so far for this gene, with the variants occurring across the entire *NF1* gene.[25] Patients with NF1 variants may manifest diverse clinical characteristics even in the families harboring the same NF1 variant,[26] indicating that genetic modifiers or environmental factors play an important role in the development of the clinical symptoms.[27-29]

In conclusion, we identified 2 novel variants and 7 reported pathogenic variants of *NF1* gene in 10 *NF1* families, which broadens the spectrum of NF1 variants. We acknowledge that this study has several limitations. Firstly, the sample size is small. Secondly, since this is a retrospective study, the clinical information collected may not have sufficient details to perform an exhaustive analysis. Hence, further research is required to elucidate the genotype-phenotype correlation.

Acknowledgement. *We would like to thank all the patients and their families who participated in this study for their cooperation. We also thank the native English speaking scientists of Elixigen Company (Huntington Beach, California) for editing our manuscript.*

References

1. Legius E, Messiaen L, Wolkenstein P, Pancza P, Avery RA, Berman Y, et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. ***Genet Med*** 2021; 23: 1506-1513.
2. Tsipi M, Poulou M, Fylaktou I, Kosma K, Tsoutsou E, Pons MR, et al. Phenotypic expression of a spectrum of neurofibromatosis type 1 (NF1) mutations identified through NGS and MLPA. ***J Neurol Sci*** 2018; 395: 95-105.
3. Kerashvili N, Gutmann DH. The management of neurofibromatosis type 1 (NF1) in children and adolescents. ***Review Expert Rev Neurother*** 2024; 24: 409-420.
4. Rana S, Low CE, Karthikeyan M, Jean Aan Koh M, Ngeow J, Chiang J. A systematic review of diagnostic modalities and strategies for the assessment of complications in adult patients with neurofibromatosis type 1. ***Cancers (Basel)*** 2024; 16: 1119.
5. Wang D, Zhang BH, Wen X, Chen KH, Xiao HT, Xu XW, et al. Clinical features and surgical treatments of scoliosis in neurofibromatosis type 1: a systemic review and meta-analysis. ***Eur Spine J*** 2024; 10.1007/s00586-024-08194-w.
6. Sarin KY, Bradshaw M, O'Mara C, Shahryari J, Kincaid J, Kempers S, et al. Effect of NFX-179 MEK inhibitor on cutaneous neurofibromas in persons with neurofibromatosis type 1. ***Sci Adv*** 2024; 10: eadk4946.
7. Mo J, Moye SL, McKay Rm, Le LQ. Neurofibromin and suppression of tumorigenesis: Beyond the GAP. ***Oncogene*** 2022; 41: 1235-1251.
8. Mabyalwa Mudau M, Dillon B, Smal C, Feben C, Honey E, Carstens N, et al. Mutation analysis and clinical profile of South African patients with neurofibromatosis type 1 (NF1) phenotype. ***Front Genet*** 2024; 15: 1331278.
9. Anastasaki C, Woo AS, Messiaen LM, Gutmann DH. Elucidating the impact of neurofibromatosis-1 germline mutations on neurofibromin function and dopamine-based learning. ***Hum Mol Genet*** 2015; 24: 3518-3528.
10. Zhu B, Zheng T, Wang W, Gu Y, Wei C, Li Q, et al. Genotype-phenotype correlations of neurofibromatosis type 1: a cross-sectional study from a large Chinese cohort. ***J Neurol*** 2024; 271: 1893-1900.
11. Han P, Wei G, Cai K, Xiang X, Deng WP, Li YB, et al. Identification and functional characterization of mutations in LPL gene causing severe hypertriglyceridaemia and acute pancreatitis. ***J Cell Mol Med*** 2020; 24: 1286-1299.
12. Zhang R, Chen R, Han P, Chen F, Kuang S, Meng Z, et al. Whole exome sequencing identified a homozygous novel variant in CEP290 gene causes Meckel syndrome. ***J Cell Mol Med*** 2020; 24: 1906-1916.
13. Ulusal SD, Gurkan H, Atli E, Ozal SA, Ciftdemir M, Tozkir H, et al. Genetic analyses of the NF1 gene in Turkish neurofibromatosis type I patients and definition of three novel variants. ***Balkan J Med Genet*** 2017; 20: 13-20.
14. Jett K, Friedman JM. Clinical and genetic aspects of neurofibromatosis 1. ***Genet Med*** 2010; 12: 1-11.
15. Riva M, Martorana D, Uliana V, Caleffi E, Boschi E, Garavelli L, et al. Recurrent NF1 gene variants and their genotype/phenotype correlations in patients with Neurofibromatosis type I. ***Genes Chromosomes Cancer*** 2022; 61:10-21.
16. Palma Milla C, Lezana Rosales JM, Lopez Montiel J, Andres Garrido LD, Sanchez Linares C, Carmona Tamajon S, et al: Neurofibromatosis type I: mutation spectrum of NF1 in spanish patients. ***Ann Hum Genet*** 2018; 82:425-436.
17. Jang MA, Kim YE, Kim SK, Lee MK, Kim JW, Ki CS. Identification and characterization of NF1 splicing mutations in Korean patients with neurofibromatosis type 1. ***J Hum Genet*** 2016; 61: 705-709.

18. Morbidoni V, Baschiera E, Forzan M, Fumini V, Ali DS, Giorgi G, et al. Hybrid minigene assay: An efficient tool to characterize mRNA splicing profiles of NF1 variants. ***Cancers (Basel)*** 2021; 13: 999.
19. Zhang J, Tong H, Fu X, Zhang Y, Liu J, Cheng R, et al. Molecular characterization of NF1 and neurofibromatosis type 1 genotype-phenotype correlations in a Chinese population. ***Sci Rep*** 2015; 5: 11291.
20. Banerjee S, Dai Y, Liang S, Chen H, Wang Y, Tang L, et al. A novel mutation in NF1 is associated with diverse intra-familial phenotypic variation and astrocytoma in a Chinese family. ***J Clin Neurosci*** 2016; 31: 182-184.
21. Banerjee S, Lei D, Liang S, Yang L, Liu S, Wei Z, et al. Novel phenotypes of NF1 patients from unrelated Chinese families with tibial pseudarthrosis and anemia. ***Oncotarget*** 2017; 8: 39695-39702.
22. Mao B, Chen S, Chen X, Yu X, Zhai X, Yang T, et al. Clinical characteristics and spectrum of NF1 mutations in 12 unrelated Chinese families with neurofibromatosis type 1. ***BMC Med Genet*** 2018; 19: 101.
23. Wang W, Qin W, Ge H, Kong X, Xie C, Tang Y, et al. Clinical and molecular characteristics of thirty NF1 variants in Chinese patients with neurofibromatosis type 1. ***Mol Biol Rep*** 2019; 46: 4349-4359.
24. Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. ***Nat Rev Cancer*** 2007; 7: 295-308.
25. Stella A, Lastella P, Viggiano L, Bagnulo R, Resta N. Clinical presentation and genetic analyses of neurofibromatosis type 1 in independent patients with monoallelic double de novo closely spaced mutations in the NF1 gene. ***Hum Mut*** 2022; 43: 1354-1360.
26. Xiao H, Yuan L, Xu H, Yang Z, Huang F, Song Z, et al. Novel and recurring disease-causing NF1 variants in two Chinese families with neurofibromatosis type 1. ***J Mol Neurosci*** 2018; 65: 557-563.
27. Cichowski K, Jacks T. NF1 tumor suppressor gene function: narrowing the GAP. ***Cell*** 2001; 104: 593-604.
28. Pemov A, Sung H, Hyland PL, Sloan JL, Ruppert SL, Baldwin AM, et al. Genetic modifiers of neurofibromatosis type 1-associated cafe-au-lait macule count identified using multi-platform analysis. ***PLoS Genet*** 2014; 10:e1004575.
29. Rojnueangnit K, Xie J, Gomes A, Sharp A, Callens T, Chen Y, et al. High incidence of Noonan syndrome features including short stature and pulmonic stenosis in patients carrying NF1 missense mutations affecting p.Arg1809: Genotype-phenotype correlation. ***Hum Mut*** 2015; 36: 1052-1063.