Clinical findings and mutational spectrum of neurofibromatosis type 1 patients in a single center of south part of Turkey

Abstract

Aim: The aim of this study is to determine the mutation spectrums and clinical characteristics of NF1 patients followed up in our center and to investigate whether there is a genotype-phenotype relationship.

Material and Methods: Sixty-three children and 34 relatives diagnosed with NF1 were included in the study. Age, gender, family history, clinical features, tumors detected in the patient at the time of diagnosis or during follow-up, orbital and cerebral magnetic resonance imaging (MRI) findings were recorded. Also results of the *NF1* gene analysis results were recorded.

Results: Fifty-three different mutations were found as a result of the *NF1* gene analysis studied from patients and their family members. Among these 53 mutations, stop codon mutation was the most frequently detected mutations. Sixteen out of 50 (32%) mutations were found to be novel mutations. Twenty-eight tumors developed in our patients. Twenty of them were optic gliomas and others were medullary thyroid carcinoma, glioblastome multiforme, pons glioma, acute lymphoblastic leukemia, pilocytic astrositoma, hypothalamic glioma, cerebral hamartoma and cardiac fibroma. No genotype-phenotype relationship was detected in patients

Conclusion: Comprehensive mutation analysis of *NF1* will increase our knowledge due to its different phenotypic characteristics even in the same mutation.

Key Words: Neurofibromatosis type 1, Genotype-phenotype correlations, mutations, novel mutations

1. Introduction

Neurofibromatosis Type I (NF1) (NF1; OMIM #162200) is a most common neurocutaneous disease showing autosomal dominant inheritance pattern (1). Two or more criteria of the National Institute of Health (NIH) below are required to diagnose NF1; (1) Six or more café-au-lait macules (CALM) (1.5 cm or larger in postpubertal individuals, 0.5 cm or larger in prepubertal individuals), (2) Two or more neurofibromas of any type or one plexiform neurofibromas, (3) Freckling in the axillary or inguinal region, (4) Optic glioma (tumor of the optic pathway), (5) Two or more Lisch nodules, (6) A distinctive bony lesion (Dysplasia of the sphenoid bone, Dysplasia or thinning of long bone cortex), (7) First-degree relative with NF1 (2). Although these criteria are used for clinical diagnosis of NF1, due to the clinical heterogeneity seen even with the same mutation, and due to the emergence of the findings over time, it may be difficult or even impossible to diagnose NF1(3).

There are also patients who cannot be confirmed genetically, although their clinical findings support NF1. On physical examination, there are diseases such as Legius syndrome, McCune Albright syndrome, mismatches repair gene defects, which can be clinically confused with NF1 at first glance with cafe-au-lait spots and some clinical findings. Therefore, it is important to confirm the diagnosis of NF1 genetically and distinguish it from other clinically similar diseases (4).

Neurofibromatosis type 1 is caused by the mutation of a gene on chromosome 17q11.2 which is responsible for production of a protein called neurofibromin that plays a role in cell signaling. *NF1* is a regulator of the *RAS* oncogene and cAMP-dependent protein kinase A (PKA)-associated signal transduction pathways, thus controlling apoptosis, cell growth, proliferation and differentiation in many human cells (5,6). NF1 is an autosomal dominant disorder which means that mutation or deletion of one allele

of *NF1* gene is sufficient for the development of NF1. GTPase-activating protein catalytic site of *NF1* gene acts as a tumor supressor gene and this may explain why the incidence of neoplasms is higher in NF1 patients.

Over 3000 pathogenic variant of *NF1* gene have been reported in the Human Gene Mutation Database (HGMD) (7). Mutations that have been observed in *NF1* gene include nonsense (stop codon), missense and splicing (intronic) mutations, deletions, insertions and rearrangements. Some of the deletions and insertions lead to frameshift mutations. About 50% of newly diagnosed NF cases have a de novo mutation. Since the *NF1* gene locus is quite large (60 exons and 350 kb of genomic DNA), it has a higher spontaneous mutation rate than most gene locus (8,9).

As a result of genomic DNA analysis by direct sequencing analysis, mutations can be detected in only 60% of patients with NF1 (10,11). While it may be possible to diagnose more patients with the next generation sequencing method used in recent years, it can also identify many de novo mutations.

In this retrospective study, we wanted to determine the diagnostic accuracy rates with next generation sequencing method, our new mutation rates according to the results of genetic analysis of patients who were clinically followed with NF1 diagnosis in our department and to investigate whether there is a genotype-phenotype correlation.

2. Material and Method

2.1. Patients

Between January 2017 and December 2019 according to the NIH criteria, 63 children and 34 relatives diagnosed with NF1 in Pediatric Oncology department were included in the study. This study was approved by the Ethics Committee of Mersin University (grant no: 2019/533). Patients' age, gender, family history, clinical features and additional NF1 findings, malignant and benign tumors detected in the patient at the

time of diagnosis or during follow-up, orbital and cerebral magnetic resonance imaging (MRI) findings were recorded. Also *NF1* gene analysis was also performed from these patients and family members, and the results were recorded.

2.2. Genetic Analysis

Molecular genetic testings were performed at Cukurova University Adana Genetic Diseases Diagnosis and Treatment Center. All gene sequence analysis was performed for *NF1* by using a peripheral blood sample. Before peripheral blood sampling written informed consent was taken from all the participants (patient/patient's family). Peripheral blood samples were collected from the patients; DNA was isolated from leukocytes using QIAsymphony DSP DNA Midi Kit (Qiaqen, Hilden, Germany) according to the manufacturer's instructions. The quality and quantity of the DNA samples was determined by Qubit™ Fluorometric Quantitation (Thermo Fisher Scientific, Waltham, MA, USA). All samples were targeted enriched for *NF1* gene (all exons and exon-intron junctions) with a custom designed kit. Amplicons were labeled with sample specific molecular barcodes for library generation. Finally, next generation sequencing was performed via Illumina Miseq (Illumina, California, USA) platform with the minimum coverage of 100x. The data sizes of FASTQ files and variant qualities were checked; bioinformatics analyses were made using QCI-A and QCI-I (Qiagen, Hildenberg, Germany). The identified variants were interpreted comparatively with 18 databases. All identified genetic alterations were categorized based on their pathogenicity according to the American College of Medical Genetics (ACMG) criteria. For patients with no detected pathogenic variants by sequencing, their samples were further analyzed using SALSA MLPA P081 and P082 NF1 Kit for exon deletions or duplications, according to the manufacturer's instructions (MRC Holland, Amsterdam,

The Netherlands). Peak areas for each separated fragment were measured using the Coffalyser software (v.140721.1958, MRC Holland).

Results

The mean age of patients (28 girls [44.4%], 35 boys [55.6%],) and first-degree relatives (13 female, 21 male) included in the study was 9.43 ± 4.58 and 28.57 ± 16.05 years, respectively. All of our patients admitted to the outpatient clinic because of CALMs on their bodies and none of them had a diagnosis of NF1 in their medical history. Similarly when the parents of the patients with a family history were questioned, it was found that there were many CALMs on their bodies, but they had not been diagnosed as NF1 until that day.

According to physical examination and family history, 45 of 63 cases included in the study were thought to be sporadic, and the remaining 18 were in the familial group. *NF1* gene mutation was found in 35 of 45 patients in the sporadic NF1 group. The clinical findings and mutation analysis results of sporadic NF1 cases are shown in Table 1. All of the patients had CLAMs. Neurofibromas, freckles in the armpit or groin areas, optic glioma, Lisch nodules and bone lesions were detected in 37.1%, 62.9%, 25.7%, 42.9% and 22.9% of the patients diagnosed with sporadic NF1, respectively. In the sporadic NF1 group, 3 patients had learning difficulties, 3 patients had epilepsy and hemangioma, scoliosis, motor retardation, fibrous cortical defect, macrophthalmia and macrocephaly, atrial septal defect, empty sella, ectopic, hypoplastic kidneys, congenital hypothyroidism, ring chromosome 18, aort coarctation, renal fusion and atrophic testis were found as additional findings in each patient. CALMs were found in the physical examination of all 52 patients from 18 families. Clinical findings of familial NF1 cases and other family members are shown in Table 2. In 18 index cases in the familial NF1 group, neurofibroma, axillary and/or inguinal freckling, optic glioma and Lisch nodule

were found to be 11.1%, 72.2%, 22.2% and 22.2%, respectively. In addition, in 34 family members in this group, neurofibroma, axillary and/or inguinal freckling, optic glioma, and Lisch nodule were detected in 34.6%, 48.1%, 11.5% and 13.5%, respectively. In the familial NF1 group, two patients had epilepsy, and vascular malformation, pseudoarthrosis, both scoliosis and learning disability, both strabismus and learning disability, and congenital melanocytic nevus were found as additional findings in each patient. In familial cases, older members of the family stated that they also had similar spots after the children were diagnosed, but none of them stated that they had been diagnosed with NF1 before.

Although ten cases showed clinical features of NF1, no mutation was detected via NGS in these patients (Table 3). MLPA assay was applied to ten patients without *NF1* mutation in genetic analysis, and two of them were found to have complete deletion in the *NF1* gene (Patient 9 and 10 in Table 3). Case number two in Table 3 admitted to our clinic with the complaint of abdominal swelling and was treated with the diagnosis of Wilms tumor. Although he had NF1 stigmas, no NF1 gene mutation was detected in the patient and he was included in the group with clinical NF1 but no mutation. After a two-year remission period, the patient was diagnosed with glioblastoma and treated. Two years after treatment for glioblastoma, she was diagnosed with T-cell acute lymphoblastic leukemia as the third primary cancer, and the patient died during treatment. A new mutation in the MSH-6 gene (c.2590G>T) was detected five years after the first admission of the patient, who was followed up with a prediagnosis of NF1, and a mismatch repair gene defect was diagnosed (12). Three patients had diffuse CALMs on physical examination and focal signal intensity areas on cranial MRI.

Twenty-eight tumors developed in our patients. Twenty of them were optic gliomas (71.42%) and others were medullary thyroid carcinoma, glioblastome multiforme, pons glioma, acute lymphoblastic leukemia, pilocytic astrositoma, hypothalamic glioma, cerebral hamartoma and cardiac fibroma. Twenty-one of these tumors (75%) were detected at the time of diagnosis and seven of them (25%) were detected during follow-up period. During the follow-up period, optic glioma developed in three patients and glioblastoma multiforme, pons glioma, acute lymphoblastic leukemia developed in one patient each. Eighteen of the patients with optic glioma were asymptomatic and detected on orbital MRI. While it was determined that there was a decrease in visual field in 4 patients with asymptomatic optic glioma in the follow-up period; symptomatic two patients presented with decreased vision. The case with medullary thyroid carcinoma was in the familial NF1 group and had germ-line homozygous *RET* proto-oncogene mutation in addition to *NF1* gene mutation (13).

Fifty-three different mutations were found as a result of the *NF1* gene analysis studied from patients and their family members. Among these 53 mutations, the stop codon was the most frequently detected mutation (28.3%, n=15). Other detected mutations are frameshift (24.5%, n=13), missense (24.5%, n=13), intronic (13.2%, n=7), deletion (3.8%, n=2), splicing (1.9%, n=1), non-sense (1.9%, n=1) and duplication (1.9%, n=1), respectively. Sixteen out of 53 (30.18%) mutations were found to be novel mutations (Table 1,2). Most of these de novo mutations were observed in sporadic cases (n=10, 62.5%). The novel mutations identified in sporadic NF1 cases were 50% frameshift (n=5), 30% stop codon (n=3), 10% missense (n=1), and 10% intronic (n=1). Among the previously identified mutations (n=23) in sporadic NF1 cases, frameshift, missense and stop codon mutations were equally detected in 27.3% (n=9), while intronic mutation was detected in 15.2% (n=5) and deletion was found to be 3% (n=1).

The novel mutations detected in famial NF1 cases were frameshift 50% (n=3) and stop codon, deletion and missense mutation were equally detected in 16.7% (n=1). Among the previously identified mutations in familial NF1 cases frameshift and stop codon mutations were equally detected in 29.4% (n=5) while missense mutation, intronic mutation and deletion was detected 23.5% (n=4), 11.8 (n=2) and 5.9% (n=1), respectively.

As seen in Table 2, although there was no NF1 finding in the mother and father, NF1 findings and *NF1* gene mutation were found in three siblings in the 6th family and in two siblings in the 13th family, and these two families were thought to have gonodal mosaicism. Again as seen in Table 2, NF1 clinical findings are present in both children and parents in families 1,3,7,8,9,10,12,14 and 18. However, the fact that *NF1* gene mutation was found only in children in these families suggested that this situation was related to somatic mosaicism.

There was no correlation between the clinical findings of our cases and the detected mutations. The same mutation was detected in families 9 and 10 in Table 2. In Table 2, three family members in the 9th family had the same clinical findings. On the other hand, one of the 10th family members had the same clinical findings as the 9th family members, while the other family member had different clinical findings.

3. Discussion

A high mutation rate is observed in *NF1* gene, while 50% of NF1 is caused by a known *NF1* gene mutation, the remaining 50% are caused by a novel mutation. The *NF1* gene has a wide range of various mutations due to its 350 kb size and high number of coding exons (14,15). Of these, 85-90% tends to be point mutations, 5-10% microdeletions, and 2% exon deletions or replications (16). It is reported that 80% of 1485 mutations identified in the literature belong to early termination codons and

truncated neurofibromine (17). It is important to perform genetic analysis in patients with NF1 diagnostic criteria, to analyze the types of mutations detected in that center, to create a national database, to identify novel mutations and to determine the genotype-phenotype relationship due to the same mutation.

In the study conducted by Varan A et al on Turkish children with NF1, the mutation rate was found to be 52% by genomic DNA analysis and they reported that frameshift mutations were the most common mutations with 38.5%. They reported that this was followed by micro deletions with 26.9% and splice site and nonsense mutations with 11.5%. It was found that 11 of 50 mutations identified in this study are novel mutations. (18). On the other hand, Ulusal SD et al. investigated mutations in DNA obtained from 24 unrelated Turkish patients and affected family members with suspected NF1, and found three novels and 12 known mutations in these individuals (19). Terzi YK et al. in a study conducted on 100 Turkish patients with a diagnosis of NF1, they identified three novel mutations (496delGT and 499delTGTT in exon 4b and 5866delA in exon 31) (20).

Sixteen of 50 (32%) mutations found in this study have not been described to date. Among them, 8 are frameshift mutations, 4 stop codon, 2 missense mutations, one intronic splice site mutation and 1 is deletion. All these mutations cause to premature terminations and truncated protein product. More than 80% of the previously described mutations have been shown to cause the truncation or shortening of the gene product, and it can be predicted that these new alterations we detected are similarly pathogenic and may affect NF1 protein function. (15).

Mutations were detected in 69 (81.17%) of a total of 85 patients, including 52 patients in 18 families and 33 patients in the sporadic NF1 group. In our study, although

there were mutations in their children and/or other family members in the familial group, no mutation was found in 11 individuals.

We think that the underlying reason for the absence of mutation in parents with these clinical findings is mosaic NF1. Mosaic NF1 occurs as a result of postzygotic *NF1* gene mutation, usually manifests itself with a milder clinical picture and is divided into somatic and gonadal mosaicism. Somatic mosaicism is caused by an *NF1* gene mutation in an early postzygotic stage. In our study nine family has a somatic mosaicism. In such cases the confirmation of the *NF1* gene mutation can only be done by molecular analysis of the affected tissues (21-24). However, in our study, no mutation was studied from the somatic DNA sample in the parents of the children who were found to have mutations, showed clinical findings and did not have a germline *NF1* mutation. If two or more children of unaffected parents develop NF1, gonadal mosaicism is suspected as a result of a postzygotic mutation in the ovum or sperm (25). In our study, *NF1* mutation was detected in two siblings without any clinical findings or mutations in the parents in two families (Family 6 and 13 in Table 2). In these families, gonadal mosaicism was considered because there was no clinical finding in the parents. Technically, it is almost impossible to show the mutation in the parents because the gonadal mosaicism needs to be studied directly from the germ cell. However, although it is difficult to determine the mutation in germ cells in the parents of these patients, it may be considered as it has important implications for genetic counseling. In our study, mutation analysis could not be performed from the germ cells of the parents suspected of having gonadal mosaicism. At the same time, additional tests could not be performed in our study because patients with suspected somatic mosaicism did not consent to tissue biopsy.

While this autosomal dominant disorder is fully expressed in adulthood, the variable clinical expressivity observed even within the same family is a common phenomenon, possibly depending on the type of the mutation that occurs in the *NF1* gene, even amongst family members. This variable clinical expression is thought to be due to allelic heterogeneity, epistaxis, and epigenetic factors such as methylation (26). Recently, genotype-phenotype correlation with microdeletion in 17q11.2, 3-bp deletion in exon 17, splice site mutation, in-frame duplication in exon 28 and 80 bp deletion have been detected (1). If a genotype-phenotype correlation is detected with the mutations detected in patients, it will be possible to predict the clinical findings that may develop in these patients and to make the necessary follow-up accurately. Although the same mutation was detected in families 9 and 10 in our study, their findings were different except learning disabilities. It has been suggested that modifying genes, epigenetic modifications, and environmental factors may be underlying factors that cause the variable phenotype in individuals with the same mutation (15,27). We think that the phenotypic difference between the two families in our study is due to this.

It is known that patients with NF1 have an increased predisposition of benign and malignant tumors compared to the healthy population (28,29). Gliomas, malignant peripheral nerve sheath tumor, leukemias, pheochromocytomas, rhabdomyosarcoma, gastrointestinal stromal tumors, breast cancers, melanomas, non-Hodgkin lymphomas and carcinoid tumors are malignant tumors associated with NF1. In a study, malignancy incidence in children with NF1 younger than 16 years reported to be 14.7% (30). Intracranial tumors and soft tissue sarcomas are the most common in children with NF1 under 10 years of age.

Central nervous tumors have been reported in approximately 20% of patients with NF1 and are usually detected in early childhood (31). Optic pathway gliomas,

which make up about 70% of these, take the first place in terms of incidence, while the brainstem gliomas take the second place with a 17% incidence (31). The incidence of OPG in NF1 can be as high as 15-20% and it can occur at any age, although it often develops before the age of 7 (32). Varan A *et al.* reported the rate of non-neurofibroma malignancy other than optic glioma as 5% in 473 patients diagnosed with NF1 (28). In this study, it was reported that soft tissue sarcomas were the most common in 26 patients with malignant tumors, followed by brain tumors. In a study by Incecik F *et al*., they reported that 19 of 120 patients with NF1 had 20 different malignancies (16.66%) and 50% of these malignancies had optic gliomas (29). Varan A *et al*. reported that 52.5% of the patients with optic glioma were patients with NF1 in a study in which they examined patients with optic glioma (33). In our series, optic glioma was the most common tumor as stated in the literature.

Mutation testing in the *NF1* gene is challenging, the detection rate of mutation is reported as less than 50%. The large size of the gene, the absence of mutation hotspots, and the presence of pseudogenes in the genome complicates mutation identification and its interpretation (34). An estimated 4.7–11% of all NF1 patients have large deletions affecting the *NF1* gene and its flanking regions at 17q11.2 (35). Large deletions of the *NF1* gene and its flanking regions are frequently associated with a severe clinical manifestation of NF1. In our series, 8 patients had clinical findings of NF1, but no mutation was detected. In patients with NF1 clinical findings and no mutations detected by sequence analysis, an appropriate method such as MLPA (Multiplex Ligation-Induced Probe Amplification) is used to detect large deletions of the *NF1* gene. MLPA analysis revealed the presence of gross deletions in 2 of these 10 patients. Whole *NF1* gene deletion was detected in both patients. Differential diagnoses of NF1 include other types of NF, diseases with one of the clinical manifestations of CALMs,

or pigment changes confused with CALMs (36). For example, constitutional mismatch repair deficiency (CMMRD) is a rare childhood cancer predisposition syndrome caused by biallelic germline mutations in one of the mismatch-repair genes. CMMRD phenotypes usually resembles to NF1 beside very high tumour risks (37). As a result of the genetic analysis performed on the development of different tumors in one of our patients who were followed up with a diagnosis of NF1 with clinical findings, although *NF1* mutation was not detected, this patient was found to be CMMRD (12). This situation makes us think that patients with clinical features of NF1 should be followed up closely for diseases included in the differential diagnosis or to perform mutation analysis again with different methods if necessary. Existing diagnostic CMMRD criteria were adapted to serve as a guideline as to when to consider CMMRD as differential diagnosis of NF1 (38). Genetic analysis was not performed in our other patients with no *NF1* mutation, as there were no findings suggestive of CMMRD.

NF1 mutation analysis is usually performed in patients with a high clinical suspicion of NF1, especially in people seeking prenatal counseling or children less than 8 years of age. To provide an accurate prenatal consultation, you need to identify the mutations in your country and know if there is a genotype-phenotype relationship. Therefore, in our study, we aimed to determine the mutations seen in our region and how these mutations are reflected in the clinic. As a result of our study, we detected novel mutations beside the known mutations, but we could not detect any genotype-phenotype correlation.

In conclusion, we consider that long-term and close follow-up of patients in a disease in which clinical findings are related with age is an important factor in determining the relationship between genotype-phenotype. We think that a long-term follow-up analysis of a large cohort of patients will contribute to a better understanding

of both the nature of NF1 and how known and novel mutations are reflected in the clinic.

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Table 1: Clinical Features and Mutational Analysis of Sporadic Neurofibromatosis Type 1 Cases

Patient	Age/Sex	Nucleotide Change	Aminoacid change	Mutation type	Symptoms
1	15, M	c.6504dupT	p.G2169fs*4	Frameshift, *Novel*	a,b,c,e,learning disability
2	1, F	c.3457_3460delCTCA	p.L1153fs*4	Frameshift	a,c,hemangioma
3	11, M	c.1185+1G>A	-	Intronic, *Novel*	a,e
4	7.5, M	c.1721G>A	p.S574N	Missense	a,c
5	5.5, F	c.7233_7234delTA	p.Q2413fs*13	Frameshift, *Novel*	a,b,c,e,
6	3, F	c.3916C>T	p.R1306*	Stop codon	a,e,motor retardation
7	9, F	c.2970_2972delAAT	p.M992del	Deletion	a,fibrous cortical defect
8	6, M	c.5305C>T	p.R1769*	Stop codon, *Novel*	a,c,d, macropthalmia, macrosephalia
9	10, M	c.5760delG	p.K1921fs*8	Frameshift, *Novel*	a,c,d,e,
10	15, F	c.3739_3742delTTTG	p.F1247fs*18	Frameshift	a,b,c,d,e,f,pilocytic astrocytoma, atrial septal defect
11	14, M	c.6858+2T>C	-	Intronic	a,b,c,empty sella
12	3.5, M	c.654+1G>T	-	Intronic	a,c,epilepsia
13	20, F	c.1845+2T>A	-	Intronic, *Novel*	a,c
14	16, M	c.3331delA	p.M1111*	Stop codon, *Novel*	a,b,c,e,
15	6.5, F	c.6976dupA	p.S2326fs*7	Frameshift, *Novel*	a
16	7, F	c.1246C>T	p.R416*	Stop codon	a,c,d
17	2, F	c.7237C>T	p.Q2413*	Stop codon	a,c
18	18, F	c.2540T>C	p.L847P	Missense	a,b,c,e,f,learning disability
19	12, F	c.2446C>T	p.R816*	Stop codon	a,b,c,f
20	8, M	c.6778G>T	p.G2260*	Stop codon, *Novel*	a,d,e,f
21	5, M	c.1466A>G	p.Y489C	Missense	a,e
22	13, F	c.6253G>A	p.V2085I	Missense	a,b,c,d,f
23	4.5, M	c.3503G>T	p.G1168V	Missense, *Novel*	a,d
24	9, F	c.2991-2A>C	-	Intronic	a,c
25	16, F	c.2939_2940delCT	p.S980*	Stop codon, *Novel*	a,b,c,d,e,f
26	9, M	c.5546G>A	p.R1849Q	Missense	a,b,e
27	4.5, M	c.2033delC	p.P678fs*10	Frameshift	a,d,ectopic and hypoplasic kidney, hipotalamic glioma
28	10, F	c.6519dupA	p.E2174fs*47	Frameshift, *Novel*	a,c,f
29	3, F	c.1721+3A>G	p.Y575C	Missense	a,c,f
30	12,M	c.1218delT	p.H407fs*5	Frameshift	a,b,d,e,learning disability
31	6, M	c.1885G>A	p.G629R	Missense	a,c,e
32	14, F	c.3046T>C	p.C1016R	Missense	a,b,c,e,epilepsia
33	9,M	c.574C>T	p.R192*	Stop codon	a,c,e
34	3, F	c.480-1G>A	-	Splicing variant	a,epilepsia,congenital hypothyroidism, ring chromosome 18
35	7,M	c.31C>T	p.Gln11Ter	Non-sense	a,b, aort coarctation,renal fusion,atrophic testis

National Institutes of Health criteria: (a)Six or more café-au-lait spots over 5mm in greatest diameter in prepubertal individuals and over 15mm in postpubertal individuals, (b)Two or more neurofibromas of any type or one or more plexiform neurofibromas, (c)Freckling in the axillary or inguinal regions, (d)Optic glioma, (e)Two or more Lisch nodules, (f)A distinctive osseous lesions such as sphenoid dysplasia or thinning of long bone cortex with or without pseudarthrosis, (g)A first-degree relative with NF-1

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Family Number	Index Case Age (year)/Sex	Family member Age (year)/Sex	Nucleotide change	Protein Change	Mutation type	Diagnostic criteria	Additional Findings
1	18, M		c.5610-2A>G	p.S891A	Intronic	a, b,c,e,g	Medullary Thyroid Carcinoma
		Brother, 15.5, M	c.5610-2A>G	p.S891A	*Somatic mosaicism*	a, b,c,e,g	
		Mother, 39, F	-	-		a, b,c,e	
2	9,F		c.1020dupT	p.V341fs*12	Frameshift	a,b,c,g	Glioblastome Multiforme
		Mother, 35, F	c.1020dupT			a,b,c	
3	6.5, F		c.60+1G>T	-	Intronic	a,g	Pseudoartroz
		Mother, 32, F	-		*Somatic mosaism*	a,b,c,g	
		Aunt, 36, F	NA			a,b,c,g	
		Cousine, 4, M	NA			a,c,g	
4	13,5, M		c.4191_9193delTGT	p.V1398del	Deletion, *Novel*	a,c,g	Pons Glioma
		Brother, 8, M	c.4191_9193delTGT	p.V1398del		a,c,g	
		Sister, 10.5, F	c.4191_9193delTGT	p.V1398del		a,c,g	Epilepsia
		Mother, 38, F	c.4191_9193delTGT	p.V1398del		a,b,c	
5	19, M		c.2326delG	p.A776fs*15	Frameshift	a,c,d,g	Cardiac Fibroma
		Father, 46, M	c.2326delG	p.A776fs*15		a,c,d	
6	12,M		c.3916C>T	p.R1306*	Stop codon	a,c,g	Epilepsia
		Brother, 15, M	c.3916C>T	p.R1306*	*Gonodal mosaicism*	a,c,g	
		Brother 8.5, M	c.3916C>T	p.R1306*		a,c,d,e,g	
7	11, M		c.6428_6432delTGTTT	p.L214fs*3	Frameshift	a,	
		Mother, 33, M	-	-	*Somatic mosaicism*		
8	9, F		c.6901_6911delGTGCTGCAG	p.V2301*	Stop codon, *Novel*	a,c,g	Acute Lymphoblastic Leukemia, Vascular malformation
		Father, 43, M	CT		*Somatic mosaicism*	a,b,c,g	
		Uncle, 40, M	-			a,b,c,g	
			NA				
9	14, M		c.3826C>T	p.R1276*	Stop codon	a,c,g	Scoliosis , Cerebral hamartoma, learning disability
		Father, 52, M	-		*Somatic mosaicism*	a,c,g	
		Uncle, 46, M	NA			a,c,g	
10	11, M		c.3826C>T	p.R1276*	Stop codon	a,c,g	Learning disability, Strabismus,
		Mother, 42, M	-		*Somatic mosaicism*	a,b,c	
11	6.5, F		c.541C>T	p.Q181*	Stop codon	a,d,g	Congenital pigmented

		Father, 36, M	c.541C>T	p.Q181*		a,b	nevus
12	10, M		c.79C>T	p.Q27*	Stop codon	a,c,d,e,g	
		Mother, 38, F	-	-	*Somatic mosaicism*	a,b	
13	8, M		c.2033dupC	p.1679fs*21	Duplication	a,c,g	
		Sister, 3, F	c.2033dupC	p.1679fs*21	*Gonodal mosaicism*	a,b,c	
14	6, F		c.6032C>T	p.A2011V	Missense, *Novel*	a,g	
		Mother, 41, F	-	-	*Somatic mosaicism*	a,c	
15	10, M		c.1733T>C	p.L578P	Missense	a,c,e,g	
		Brother, 7, M	c.1733T>C	p.L578P		a,c,d,e,g	
		Mother, 36, F	c.1733T>C	p.L578P		a,b	
16	10, M		c.2903T>G	p.M968R	Missense	a,c,g	Hydronephrosis
		Sister, 4, F	c.2903T>G	p.M968R		a,d,g	
		Father, 38, M	c.2903T>G	p.M968R		a,b,c,g	
		Uncle, 35, M	c.2903T>G	p.M968R		a,b,g	Strabismus
		Cousine, 12, F	c.2903T>G	p.M968R		a,c,d,e,g	Strabismus
		Cousine12, M	c.2903T>G	p.M968R		a,c,d,e,g	
		Grandmother, 62, F	NA	NA		a,b	
17	9, F		c.1658A>G	p.H553R	Missense	a,d,g	
		Father, 33, M	c.1658A>G	p.H553R		a,b,c,g	
		Uncle, 30, M	NA	NA		NA	
		Cousine, 7, M	NA	NA		NA	
18	12, M		c.6428_6432delTGTTT	p.L214fs*3	Frameshift	a,c,e,g	
		Mother, 34, M	-	-	*Somatic mosaicism*	a,b,c,e	

National Institutes of Health criteria: a) Six or more café-au-lait spots over 5mm in greatest diameter in prepubertal individuals and over 15mm in postpubertal individuals. b) Two or more neurofibromas of any type or one or more plexiform neurofibromas. c) Freckling in the axillary or inguinal regions. d) Optic glioma. e) Two or more Lisch nodules. f) A distinctive osseous lesions such as sphenoid dysplasia or thinning of long bone cortex with or without pseudarthrosis. g) A first-degree relative with NF-1, NA: Not Available

Table 3: Clinical Features of Neurofibromatosis Type 1 Cases with No Mutation Detected

Patient	Age (years)/Sex	Diagnostic Criteria	Additional findings
1	14, F	a,c	Milral valve prolapsus, focal areas of the signal intensity in cranial MRI
2	2, M	a,c	Wilms tumor, Glioblastoma, ALL
3	8, M	a,b,c,g	-
4	11, M	a	Focal areas of the signal intensity in cranial MRI
5	6, F	a,c,e	-
6	13, M	a,c,e,g	-
7	7, M	a	Epilepsia, focal areas of the signal intensity in cranial MRI
8	5.5, F	a,g	-
9	16, F	a,c,e	Focal areas of the signal intensity in cranial MRI
10	2, M	a,c,e	Focal areas of the signal intensity in cranial MRI

National Institutes of Health criteria: (a) Six or more café-au-lait spots over 5mm in greatest diameter in prepubertal individuals and over 15mm in postpubertal individuals, (b) Two or more neurofibromas of any type or one or more plexiform neurofibromas, (c) Freckling in the axillary or inguinal regions, (d) Optic glioma, (e) Two or more Lisch nodules, (f) A distinctive osseous lesions such as sphenoid dysplasia or thinning of long bone cortex with or without pseudarthrosis, (g) A first-degree relative with NF-1