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Original Article

The Spectrum of *NF1* Gene Variations in Southeastern Turkey

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ABSTRACT

Aim: We aimed to expand the variant spectrum of the *NF1* gene in Southeastern Turkey. Neurofibromatosis type 1 (NF1) disease is an inherited skin disorder with variable severity and heterogeneous systemic involvement. The pathogenic variations of the *NF1* gene are responsible for the *NF1* phenotype.

Materials and Methods: In this study, clinical and molecular manifestations of 92 molecularly confirmed NF1 patients from 86 unrelated families are presented. The next-generation sequencing method (using Ion Torrent PGM™ Platform) was performed to analyze all coding exons of the NF1 gene.

Results: Seventy-six different *NF1* variations were identified with 27 of them being novel. 42.5% of the patients were familial and 57.5% were sporadic. Except for one 20-year-old patient with c.1637dupT variant who presented with pilocytic astrocytoma without cutaneous findings, all the other patients demonstrated several typical clinical criteria of NF1.

Conclusion: Although NF1 diagnostic criteria are the most widely used and proficient clinical diagnostic tool, *NF1* gene analysis can be applied as a definitive diagnostic tool in cases with atypical presentations and in early childhood.

Keywords: NF1, next-generation sequencing, Southeastern Turkey

Introduction

Neurofibromatosis type 1 (NF1; OMIM 162200) is one of the most common hereditary disorders. It is inherited as an autosomal dominant trait. The estimated incidence at birth is 1/3,000 (1,2). Multiple café-au-lait spots, axillary/inguinal freckling, multiple cutaneous neurofibromas, iris Lisch nodules, and choroidal freckling are the characteristic features of NF1 (3). NF1 also manifests as multisystemic disorders with musculoskeletal, vascular, central nervous, and peripheral nervous system involvement such as scoliosis, tibial dysplasia, vasculopathy, glioma, and malignant peripheral nerve sheath tumors (4).

Clinical diagnosis of NF1 is based on the National Institutes of Health (NIH) diagnostic criteria (5). However, without a family history, these criteria may be insufficient in early childhood as most of the clinical features manifest later in life (3).

The NF1 phenotype is caused by germline heterozygous pathogenic variants of the *NF1* gene. NF1 is located at chromosome 17q11.2 and coded neurofibromin 1 protein that acts as a regulator of Ras activity. NF1, one of the largest genes in the human genome, consists of 57 coding exons and 12,362 base pairs transcript length (transcript reference, NM_000267.3).

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In recent years, next-generation sequencing (NGS) technology has enabled many genes, regardless of size, to be analyzed systematically, comprehensively, more easily and more cost-effectively. Most studies demonstrated that NGS with in-solution hybridization-based enrichment

provides a high mutation detection rate comparable to that of conventional direct capillary sequencing methods for the molecular diagnosis of neurofibromatosis. In this study, we aimed to present the variant spectrum of NF1 patients from the region of Southeastern Turkey and investigate if there is a clear genotype-phenotype correlation (6).

Materials and Methods

Profile of the Patients

The clinical and genetic data of the NF1 patients who were referred to the Medical Genetics Clinic, Gaziantep Ersin Arslan Training and Research Hospital between May 2016 and December 2019 were evaluated retrospectively. Peripheral blood samples were obtained after taking informed consent from all participants or the legal guardians of those children under the age of 18. The study was approved by the Ethics Committee of the Gaziantep University Medical Faculty (approval number: 65587614-774.99-291, date: 04/10/2017).

Ninety-two Turkish patients from 86 unrelated families who were both clinically and molecularly diagnosed with NF1 were included in this study. Fifty-four (58.7%) patients were male and thirty-eight (41.3%) were female. Age at diagnosis was in the range of 1-46 years.

The clinical diagnosis was performed based on the presence of two or more of the diagnostic criteria proposed by the NIH Consensus Development Conference (5). The diagnosis of NF1 was established in patients who had two or more of the following NIH criteria: Six or more cafe-au-lait macules (one of them must be greater than 5 mm and 15 mm, prepubertal and post-pubertal respectively); two or more neurofibromas or 1 plexiform neurofibroma; freckling in the axillary or inguinal regions; optic glioma; two or more Lisch nodules; a distinctive osseous lesion (sphenoid dysplasia or tibial pseudarthrosis); and a first-degree relative with neurofibromatosis type 1.

Genetic Analysis

Genomic DNA was extracted from whole blood samples using an automated method (RSC whole blood DNA kit) in the Maxwell® 16 (Promega Corporation, Madison, WI). NF1 (57 coding exons, NM_000267.3) amplicons were designed using the AmpliSeq Designer software (Life Technologies, CA, USA), targeting the complete coding sequence of the *NF1* gene by 120 amplicons. The design target coverage was 99.49%.

Amplicon library was prepared using the Ion AmpliSeq Library Kit Plus, Xpress Barcode Adapters 1-96 Kit (Thermo Fisher Scientific), then pooled together using Qubit 1X dsDNAhs Assay kit and Qubit 4 Fluorometer (Thermo Fisher Scientific). Emulsion PCR, and Ion Sphere Particles enrichment were carried out in the Ion Chef System, then loaded into an Ion 530 chip. NGS was performed via Ion 510 & Ion 520 & Ion 530 kit-Chef (Thermo Fisher Scientific). Data were processed using Ion Torrent Suite Software (Thermo Fisher Scientific) and Ion Reporter Software (Thermo Fisher Scientific).

The Human Gene Mutation Database (HGMD) (7) and Leiden Open Variation Database (LOVD) were used to determine whether a variant was novel or not. Several prediction algorithms, including SIFT (http://sift.jcvi.org), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), Human Splicing Finder (http://www.umd.be/HSF/) and Mutation taster (http://www.mutationtaster.org) were used to determine any damaging effects on the protein. The Genome Aggregation Database (https://gnomad.broadinstitute.org) was used to estimate the minor allele frequency score.

Nomenclature of the variants was based on the NM_000267.3 (NCBI transcript number). Variants were reviewed using dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). A minimum 30X coverage for all bases was accepted for a reliable variant calling. Ion Reporter version 5.0 software was used to annotate variants. Integrated Genomics Viewer (http://software.broadinstitute.org/software/igv/) was used for visual assessment of the revealed variants.

Novel variants are reported to the Human Genome Variation Society guidelines and checked by using Mutalyzer tool (https://mutalyzer.nl/about). All variants were classified by using The American College of Medical Genetics and Genomics (ACMG) guidelines criteria (8). Some variants were validated with direct capillary sequencing that was performed by using the BigDyeTerminator kit v3.1 (LifeTechnologies, Darmstadt, Germany) and an automated capillary sequencer (3500xl Genetic Analyzer, Life Technologies). The obtained sequence data were analyzed using the Seq-Scape (Ver. 2.1) program (Applied Biosystems).

Results

Clinical Manifestations of the Patients

We reviewed the clinical data in 73 of the 92 patients. The frequencies of clinical features are sorted by age ranges in Table I. The median age was 8 years. 42.5% of patients were familial and 57.5% were sporadic cases. Seventy-one (97.2%) patients were suffering from café-au-lait spots. Axillary or inguinal freckling was present in 27 patients (36.9%). The other common skin manifestation were cutaneous neurofibromas that accounted for 19.1% (14/73) of cases. Optic glioma was identified in 6.8% (5/73) of the patients. Hamartomas were detected with magnetic resonance imaging in 22 of the patients (30.1%) (Table II).

Characterization of the NF1 Variants

Seventy-six variations of which two were probably somatic were detected in 92 patients from unrelated families. The identified variants were as follows: 36 frameshift variants (41.9%) resulting from small insertions, deletions or indels; 27 non-sense variations (31.4%); 14 missense variants (16.2%); 6 splicing alterations (7%), and 3 in-frame deletions or indels (3.5%) (Figure 1).

Twenty-seven (35%) of the variants were novel and had not been previously reported in HGMD or LOVD. The c.2446C>T, c.3826C>T, c.5839C>T, c.2546dupG showed familial segregation. The c.5107C>T (15%), c.3721C>T (20%) variants were detected at lower fraction percentages; 15% and 20% respectively. The c.2033dupC, c.2446C>T, c.3525_3526delAA, c.3826C>T, c.4084C>T, c.5546G>A, c.6792C>G variations were identified in more than one unrelated family (Table III). Only one of the variations (c.7674G>A) was interpreted as a variant of unknown origin (VUS) based on ACMG criteria. The other three were likely pathogenic and 23 of them were pathogenic. 24 variants produced truncated protein as a result of premature stop codon, which is a significant indication of their pathogenicity. All novel variants were predicted to be deleterious by at least one in-silico analysis.

Discussion

As *NF1* is the one of the most common inherited disorders and *NF1* is one of the largest genes in the human genome, a great number of variations (over 3,000) have been reported in HGMD to date. Even though previously reported pathogenic variation types show diversity, most of them cause severe truncated gene products (9). Most of the pathogenic variants (93%) are small nucleotide alterations (including non-sense, missense, insertion or deletion) and splicing variants. Intragenic deletions/duplications (2%) and microdeletions (5%) are rarely detected (10). In this study, we identified 76 different mutations in 92 families. Most of the variant types are frameshift, non-sense and splice-site, similar to the literature (10-12). The missense variation rate was relatively high (14 variants, 16%) in accordance with similar studies (11-13). There were some conflicting pathogenicity variants such as c.7674G>A and c.2764G>A which were interpreted as VUS based on ACMG criteria. The patient with c.7674G>A had a typical NF1 phenotype, which was a strong indicator showing the variant's pathogenicity. However, the case with c.2764G>A variant did not reach the optimum clinical registry.

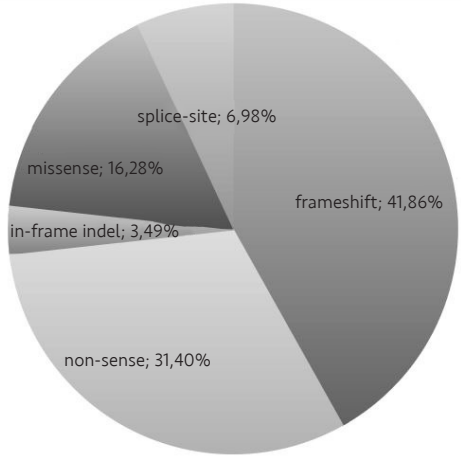

Figure 1. Type of NF1 variations

Table I. Range of the clinical features according to the age groups

Age		6>CALs	Freckling	Neurofibroma	Optic glioma	Lisch nodule	Hamartoma
Year	**n**	**n (%)**					
0-1	15	15 (100)	2 (13.3)	2 (13.3)	2 (13.3)	5 (33.3)	4 (26.6)
2-4	13	13 (100)	4 (30.7)	1 (7.6)	2 (15.3)	4 (30.7)	5 (38.4)
5-18	34	34 (100)	15 (100)	4 (11.7)	1 (12.9)	6 (17.6)	12 (35.2)
19-30	4	3 (75)	3 (44.1)	1 (25)	0 (0)	0 (0)	0 (0)
31-60	7	6 (85.7)	3 (75)	6 (87.5)	0 (0)	2 (28.5)	1 (14.2)

n: Number; CALs: Cafe au lait spots

Table II. Clinical data of the patients

Family	Case	Sex	Age (yr)	Variation (codon number)	Family history	CALs	Neurofibromas	Freckling	Optic glioma	Lisch nodules	Hamartoma	Other clinical findings
F1	C1	M	1	c.1756_1759del	-	+	-	-	-	-	N/R	-
F2	C2	M	13	c.5546G>A	+	+	-	-	-	-	+	-
F3	C3	M	15	c.3525_3526delAA	N/R	N/R	N/R	N/R	N/R	N/R	+	N/R
F4	C4	F	1	c.3739delT	-	+	-	-	+	-	+	-
F5	C5	F	11	c.1019_1020delCT	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F6	C6	M	2	c.1393-9T>A	+	+	-	N/R	-	-	-	N/R
F7	C7	F	1	c.1428_1431delATTTinsCC	+	+	-	N/R	-	-	-	-
F8	C8	M	7	c.1466A>G	-	N/R	-	-	-	-	-	-
F9	C9	F	13	c.1557dupA	+	+	-	N/R	-	-	N/R	-
F10	C10	M	45	c.1697delC	-	+	+	N/R	N/R	N/R	-	-
F11	C11	M	41	c.1721+3A>C	+	-	+	N/R	N/R	N/R	N/R	-
F12	C12	F	11	c.2446C>T	+	+	+	+	-	-	+	-
F12	C13	M	6	c.2446C>T	+	+	N/R	-	N/R	N/R	-	Short stature
F12	C14	F	28	c.2446C>T	+	+	-	+	N/R	N/R	-	-
F13	C15	F	11	c.2446C>T	-	+	+	+	-	-	N/R	-
F14	C16	F	11	c.2970_2972delAAT	-	+	-	N/R	-	-	-	-
F15	C17	M	1	c.3826C>T	-	+	+	N/R	+	+	+	Seizure
F16	C18	F	2	c.3826C>T	+	+	-	-	-	-	-	-
F16	C19	F	1	c.3826C>T	+	+	-	-	-	+	-	-
F16	C20	M	38	c.3826C>T	+	+	+	+	-	+	N/R	-
F17	C21	F	8	c.3916C>T	-	+	-	+	-	+	+	-
F18	C22	M	10	c.4075_4076delinsAA	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F19	C23	F	9	c.4084C>T	+	+	+	+	-	+	+	Seizure
F20	C24	M	35	c.4084C>T	-	+	+	-	-	+	-	-
F21	C25	F	9	c.4267A>G	-	+	-	+	+	+	-	Asthma
F22	C26	M	35	c.4537C>T	+	+	-	+	N/R	-	N/R	Arrhythmia
F23	C27	M	9	c.4621delC	-	+	-	+	-	+	+	-
F24	C28	F	2	c.4822_4826delCTGAC	+	+	N/R	+	-	-	-	-
F25	C29	M	5	c.5522T>A	-	+	-	+	-	+	-	N/R
F26	C30	M	1	c.5722G>T	-	+	-	+	N/R	+	N/R	-
F27	C31	M	12	c.5839C>T	+	+	-	+	-	+	+	N/R
F27	C32	F	8	c.5839C>T	+	+	-	+	-	-	N/R	-
F28	C33	M	3	c.6334_6335delCT	-	+	-	-	-	-	-	N/R
F29	C34	M	1	c.6791dupA	-	+	-	-	-	+	+	-
F30	C35	M	1	c.6792C>G	-	+	-	-	-	-	-	N/R
F31	C36	F	13	c.6792C>G	-	+	-	+	-	-	+	-
F32	C37	F	10	c.6792C>G	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R

Table II. continued

F33	C38	F	5	c.7206_7207delCA	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F34	C39	M	25	c.7237C>T	-	+	-	+	-	-	-	-
F35	C40	M	10	c.7285C>T	-	+	-	-	-	-	-	-
F36	C41	M	29	c.7419G>A	+	+	+	+	-	-	-	-
F37	C42	M	9	c.7486C>T	-	+	N/R	N/R	-	-	-	N/R
F38	C43	F	9	c.953_956delAAAG	-	+	-	N/R	N/R	N/R	-	Scoliosis
F39	C44	M	10	c.1548dupC	+	+	-	yok	-	-	-	-
F40	C45	M	13	-	-	+	-	N/R	-	-	+	-
F41	C46	M	46	c.2867_2868delCCinsA	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F42	C47	M	3	c.2890dupA	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F43	C48	F	1	c.3457_3460delCTCA	-	+	-	-	-	-	-	N/R
F44	C49	F	2	c.3525_3526delAA	+	+	-	-	-	-	-	N/R
F45	C50	M	8	c.5844_5845delAA	-	+	-	+	-	-	-	N/R
F46	C51	M	4	c.7674G>A	-	+	-	+	-	+	+	-
F47	C52	F	3	c.2033dupC	+	+	N/R	N/R	-	-	N/R	-
F48	C53	F	7	c.1381C>T	-	+	-	N/R	-	-	+	-
F49	C54	M	4	c.1261-3_1270del TAGTCCGCATTGG	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F50	C55	F	3	c.1318C>T	-	+	-	+	-	-	+	-
F51	C56	F	16	c.1721G>A	N/R	N/R	N/R	N/R	+	N/R	-	N/R
F52	C57	M	8	c.2546dupG	+	+	-	-	-	+	-	-
F52	C58	M	1	c.2546dupG	+	+	+	+	-	+	+	-
F53	C59	F	2	c.3058delG	+	+	-	+	+	-	+	Skeletal deformity
F54	C60	F	13	c.3114-2A>G	-	+	-	-	-	-	-	-
F55	C61	M	6	c.3610C>G	-	+	-	-	-	-	+	Seizure
F56	C62	M	8	c.5003_5004insGGTA	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F57	C63	M	3	c.6263delT	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F58	C64	M	8	c.4288A>G	-	+	-	+	-	-	N/R	N/R
F59	C65	M	36	c.6125delT	+	+	+	+	-	-	+	-
F60	C66	M	12	c.1496T>G	+	+	-	N/R	-	-	-	-
F61	C67	F	4	c.1737_1738delTT	+	+	1 +	-	-	+	-	-
F62	C68	M	21	c.1748A>G	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F63	C69	F	6	c.1885G>A	+	+	-	N/R	-	-	-	-
F64	C70	F	16	c.2033dupC	+	+	N/R	N/R	N/R	N/R	N/R	N/R
F65	C71	M	13	c.2033dupC	+	+	-	+	-	-	-	-
F66	C72	F	13	c.2097dupC	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F67	C73	M	9	c.2252-3T>G	+	+	N/R	N/R	N/R	N/R	+	Seizure
F68	C74	M	1	c.2325+3A>G	-	+	-	-	-	-	-	N/R
F69	C75	F	1	c.2604delT	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F70	C76	M	5	c.2764G>A	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F71	C77	F	13	c.3470T>A	-	+	N/R	N/R	N/R	N/R	N/R	N/R

Table II. continued

F72	C78	M	2	c.446dupA	-	+	N/R	-	+	+	+	Skeletal deformity
F73	C79	M	10	c.484C>T	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F74	C80	F	9	c.4817T>A	+	+	-	+	-	-	-	Scoliosis
F75	C81	F	1	c.5719G>T	-	+	-	-	-	-	-	-
F76	C82	M	1	c.7229delT	-	+	-	-	-	-	-	-
F77	C83	M	2	c.7518_7519delGCinsCT	+	+	-	-	-	-	-	-
F78	C84	F	1	c.1404dupT	-	+	-	-	N/R	N/R	-	-
F79	C85	M	20	c.1637dupT	-	-	-	-	-	-	-	Pilositic astrocytoma
F80	C86	F	13	c.1756_1759delACTA	-	+	+ 1	+	-	-	N/R	-
F81	C87	M	21	c.3721C>T	N/R	N/R	N/R	N/R	N/R	N/R	-	N/R
F82	C88	F	8	c.3871delG	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
F83	C89	M	7	c.4816_4817insGG	N/R	+	-	-	-	-	+	N/R
F84	C90	M	1	c.5107C>T	-	+	-	-	-	-	N/R	-
F85	C91	M	4	c.5546G>A	N/R	+	-	N/R	-	+	+	Speech delay
F86	C92	M	38	c.5827delG	-	+	+	-	-	-	-	-

N/R: Not reported, CALs: Cafe au lait spots, M: Male, F: Female
C3-C5-C22-C37-C38-C46-C47-C54-C56-C57-C62-C63-C68-C72-C75-C76-C79-C87-C88 are out of clinical table (Table 1)

There is not a well-recognized hot spot region in the *NF1* gene (14-16). In this study, we found only one recurrent variant (c.6792C>G) in two unrelated families. It did not show significant variant aggregation in any exon (Figure 2). Although we observed relative variant density in exon 13 according to the exon length (Figure 2), it was insufficient to speculate that exon 13 is a hotspot region. Moreover, the variant frequency was lower both in the first and last few exons which code the constitutional amino acids. This can be attributed to the variants on the distal part of the gene being less effective in causing the *NF1* phenotype.

Six functional domains were determined on the *NF1* protein: The cysteine and serine rich domain (CSRD; exons 1-22), the tubulin binging domain (TBD; exons 22-27), the domain responsible for interactions with RAS and GTP hydrolysis (GRD; exons 27-34), the bipartite lipid binging domain (first part) (Sec14; exons 35-36), the bipartite lipid binging domain (second part) (PH; exons 35-36), and the carboxyl-terminal domain (CTD; exons 37-52) (17). The distribution of the variants according to functional domains were CSRD (31/76-40.7%), TBD (9/76-11.8%), GRD (11/76-14.4%) Sec 14 and PH (5/76-6.5%), and CTD (20/76-26.3%). We did not find any variants outside of the functional domains.

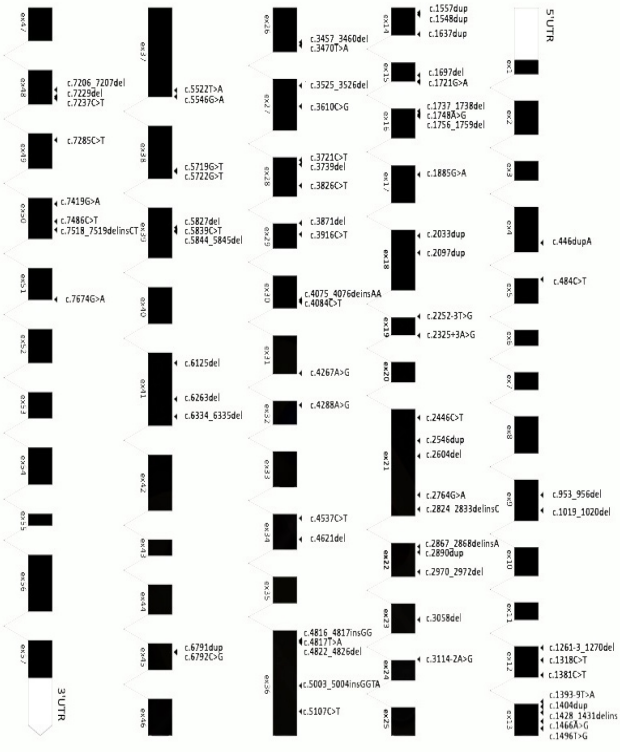

Figure 2. Distribution of the variants according to functional domains

Table III. Distribution of the identified NF1 variants

Family	Variation (codon number)	Protein number	HGMD reference	LOVD	Type	Predicted effect	ACMG criteria	ACMG prediction	Exon
F1, F80	c.1756_1759del	p.Thr586Valfs*18	CD982825	R	Deletion	Frameshift	PVS1, PM1, PM2 PP3, PP5	P	16
F2, F85	c.5546G>A	p.Arg1849Gln	CM1718194	R	Substitution	Missense	PM2, PP2, PP3, PP5	LP	37
F3, F44	c.3525_3526delAA	p.Arg1176Serfs*18	CD000971	R	Deletion	Frameshift	PVS1, PM1, PM2, PP3, PP5	P	27
F4	c.3739delT	p.Phe1247Leufs*19	Novel	N/R	Deletion	Frameshift	PVS1, PM1, PM2, PP3	P	28
F5	c.1019_1020delCT	p.Ser340fs	CD972347	R	Deletion	Frameshift	PVS1, PM1, PM2, PP3, PP5	P	9
F6	c.1393-9T>A	-	CS000058	R(P)	Substitution	Splice site	PM2	VUS	12-13
F7	c.1428_1431delATTTinsCC	p.Lys476Asnfs*14	Novel	N/R	Deletion	Frameshift	PVS1, PM2, PP3	P	13
F8	c.1466A>G	p.Tyr489Cys	CM1111787	R(P)	Substitution	Missense	PM1, PM2, PP2, PP3, PP5	P	13
F9	c.1557dupA	p.Gly520fs	Novel	N/R	Duplicaton	Frameshift	PVS1, PM1, PM2 PP3	P	14
F10	c.1697delC	p.Pro566Leufs*2	CD1815862	N/R	Deletion	Frameshift	PVS1, PM1, PM2, PP3	P	15
F11	c.1721+3A>C	-	CS941514	R(P)	Substitution	Splice site	PM2	VUS	15-16
F12, F13	c.2446C>T	p.Arg816*	CM971040	R(P)	Substitution	Non-sense	PVS1, PM1, PM2, PP3, PP5	P	21
F14	c.2970_2972delAAT	p.Met991del	CD931025	R(P)	Deletion	In frame deletion	PM1, PM2, PM4, PP3, PP5	P	22
F15, F16	c.3826C>T	p.Arg1276*	CM950847	R(P)	Substitution	Non-sense	PVS1, PS3, PM1, PM2 PP3	P	28
F17	c.3916C>T	p.Arg1306*	CM981381	R(P)	Substitution	Non-sense	PVS1, PM1, PM2, PP3, PP5	P	29
F18	c.4075_4076delinsAA	p.Pro1359Asn	Novel	N/R	Indel	In-frame indel	PM1, PM2, PP3, PP5	LP	30
F19, F20	c.4084C>T	p.Arg1362*	CM971046	R(P)	Substitution	Non-sense	PVS1, PM1, PM2, PP3, PP5	P	30
F21	c.4267A>G	p.Lys1423Glu	CM920506	R(P)	Substitution	Missense	PM1, PM2, PM5, PP2, PP3, PP5	P	31
F22	c.4537C>T	p.Arg1513*	CM941093	R(P)	Substitution	Non-sense	PVS1, PM1, PM2, PP3, PP5	P	34
F23	c.4621delC	p.Leu1541fs	Novel	R(P)	Deletion	Frameshift	PVS1, PM1, PM2, PP3, PP5	P	34

Table III. continued

F24	c.4822_4826delCTGAC	p.Leu1608fs	Novel	N/R	Deletion	Frameshift	PVS1, PM1, PM2, PP3	P	36
F25	c.5522T>A	p.Leu1841*	Novel	N/R	Substitution	Non-sense	PVS1, PM1, PM2, PP3	P	37
F26	c.5722G>T	p.Glu1908*	CM143452	R(P)	Substitution	Non-sense	PVS1, PM1, PM2, PP3	P	38
F27	c.5839C>T	p.Arg1947*	CM900173	R(P)	Substitution	Non-sense	PVS1, PM1, PM2, PP3, PP5	P	39
F28	c.6334_6335delCT	p.Leu2112Valfs	CD1415205	R(P)	Deletion	Frameshift	PVS1, PM1, PM2, PP3, PP5	P	41
F29	c.6791dupA	p.Tyr2264*fs	CI962317	R(P)	Duplicaton	Frameshift	PVS1, PM1, PM2, PP3	P	45
F30, F31, F32	c.6792C>G	p.Tyr2264*	CM972796	R(P)	Substitution	Non-sense	PVS1, PM1, PM2, PP3, PP5	P	45
F33	c.7206_7207delCA	p.His2402Glnfs*4	CD031873	R(P)	Deletion	Frameshift	PVS1, PM1, PM2, PP3, PP5	P	48
F34	c.7237C>T	p.Gln2413*	CM000817	N/R	Substitution	Non-sense	PVS1, PM1, PM2, PP3	P	48
F35	c.7285C>T	p.Arg2429*	CM000818	R(P)	Substitution	Non-sense	PVS1, PM1, PM2, PP3, PP5	P	49
F36	c.7419G>A	p.Trp2473*	Novel	N/R	Substitution	Non-sense	PVS1, PM1, PM2, PP3, PP5	P	50
F37	c.7486C>T	p.Arg2496*	CM941096	R(P)	Substitution	Non-sense	PVS1, PM2, PP3, PP5	P	50
F38	c.953_956delAAAG	p.Glu318Valfs*57	CD1512843	R(P)	Deletion	Frameshift	PVS1, PM1, PM2, PP3	P	9
F39	c.1548dupC	p.Glu517Argfs*41	Novel	N/R	Duplicaton	Frameshift	PVS1, PM1, PM2, PP3	P	14
F40	c.2824_2833delAGCAAGTTTTinsC	-	Novel	N/R	Indel	In-frame indel	PM1, PM2, PM4, PP3	LP	21
F41	c.2867_2868delCCinsA	p.Thr956Lysfs	Novel	N/R	Indel	Frameshift	PVS1, PM1, PM2, PP3	P	22
F42	c.2890dupA	p.Thr964Asnfs*11	Novel	N/R	Duplicaton	Frameshift	PVS1, PM1, PM2 PP3	P	
F43	c.3457_3460delCTCA	p.Leu1153Metfs	CD972351	R(P)	Deletion	Frameshift	PVS1, PM1, PM2, PP3, PP5	P	26
F45	c.5844_5845delAA	p.Arg1949Serfs*6	CD941733	R(P)	Deletion	Frameshift	PVS1, PM1, PM2, PP3, PP5	P	39
F46	c.7674G>A	p.Met2558Ile	Novel	N/R	Substitution	Missense	PM2, PP2	VUS	51
F47, F64, F65	c.2033dupC	p.Ile679Aspfs*10	CI951961	R(P)	Duplicaton	Frameshift	PVS1, PM1, PM2, PP3, PP5	P	18
F48	c.1381C>T	p.Arg461*	CM000780	R(P)	Substitution	Non-sense	PVS1, PM2, PP3, PP5	P	12

Table III. continued

F49	c.1261-3_1270del TAGTCCGCATTGG	-	Novel	N/R	Deletion	Splice site	PVS1, PM2, PP3	P	12
F50	c.1318C>T	p.Arg440*	CM950845	R(P)	Substitution	Non-sense	PVS1, PM1, PM2, PP3, PP5	P	12
F51	c.1721G>A	p.Ser574Asn	CM062898	R(P)	Substitution	Missense	PM1, PM2, PM5, PP2, PP3, PP5	P	15
F52	c.2546dupG	p.p.Val850Serfs*15	CI098059	R(P)	Duplicaton	Frameshift	PVS1, PM1, PM2, PP3	P	21
F53	c.3058delG	p.Glu1020Lysfs*2	Novel	N/R	Deletion	Frameshift	PVS1, PM1, PM2, PP3	P	23
F54	c.3114-2A>G	-	CS147208	R(P)	Substitution	Splice site	PVS1, PM2, PP3, PP5	P	23-24
F55	c.3610C>G	p.Arg1204Gly	CM973234	R(P)	Substitution	Missense	PM1, PM2, PM5, PP2, PP3, PP5	P	27
F56	c.5003_5004insGGTA	p.Tyr1668*	Novel	N/R	Insertion	Non-sense	PVS1, PM1, PM2, PP3	P	36
F57	c.6263delT	p.Phe2088Serfs*2	CD1719515	N/R	Deletion	Frameshift	PVS1, PM1, PM2, PP3, PP5	P	41
F58	c.4288A>G	p.Asn1430Asp	CM113590	R(P)	Substitution	Missense	PM1, PM2, PM5, PP2, PP3, PP5	P	32
F59	c.6125delT	p.Leu2042Tyrfs*7	Novel	N/R	Deletion	Frameshift	PVS1, PM2, PP3	P	41
F60	c.1496T>G	p.Leu499Arg	CM1512946	R(P)	Substitution	Missense	PM1, PM2, PP2, PP3, PP5	LP	13
F61	c.1737_1738delTT	p.Phe579Leufs*8	Novel	N/R	Deletion	Frameshift	PVS1, PM1, PM2 PP3	P	16
F62	c.1748A>G	p.Lys583Arg	CM1111788	R(P)	Substitution	Missense	PM1, PM2, PP2, PP5, BP4	LP	16
F63	c.1885G>A	p.Gly629Arg	Novel	R(P)	Substitution	Missense	PS1, PS3, PM1, PM2, PP2, PP3	P	17
F66	c.2097dupC	p.Thr700Hisfs*2	Novel	N/R	Duplicaton	Frameshift	PVS1, PM2, PP3	P	18
F67	c.2252-3T>G	-	CS086414	N/R	Substitution	Splice site	PM2, PP5	VUS	18-19
F68	c.2325+3A>G	-	CS1311513	R(P)	Substitution	Splice site	PM2, PP5	VUS	19-20
F69	c.2604delT	p.Pro869Glnfs*9	CD153889	R(P)	Deletion	Frameshift	PVS1, PM1, PM2	P	21
F70	c.2764G>A	p.Gly922Ser	CM1719434	R(P)	Substitution	Missense	PM2, PP2, PP3	VUS	21
F71	c.3470T>A	p.Val1157Glu	Novel	N/R	Substitution	Missense	PM1, PM2, PP3, PP5	LP	26
F72	c.446dupA	p.Asn149Lysfs*7	Novel	N/R	Duplicaton	Frameshift	PVS1, PM2, PP3	P	4
F73	c.484C>T	p.Gln162*	CM073223	R(P)	Substitution	Non-sense	PVS1, PM1, PM2 PP3, PP5	P	5

Table III. continued

F74	c.4817T>A	p.Val1606Asp	CM1919720	N/R	Substitution	Missense	PM1, PM2, PP3, PP5	LP	36
F75	c.5719G>T	p.Glu1907*	CM043552	N/R	Substitution	Non-sense	PVS1, PM1, PM2 PP3, PP5	P	38
F76	c.7229delT	p.Val2410Glyfs*9	Novel	N/R	Deletion	Frameshift	PVS1, PM1, PM2	P	48
F77	c.7518_7519delGCinsCT	p.Gln2507*	Novel	N/R	Indel	Non-sense	PVS1, PM1, PM2 PP3	P	50
F78	c.1404dupT	p.Lys469*	Novel	N/R	Duplicaton	Non-sense	PVS1, PM1, PM2 PP3	P	13
F79	c.1637dupT	p.Met546Ilefs*12	Novel	N/R	Duplicaton	Frameshift	PVS1, PM2, PP3	P	14
F81	c.3721C>T	p.Arg1241* (%20)	CM000799	R(P)	Substitution	Non-sense	PVS1, PM1, PM2, PP3, PP5	P	28
F82	c.3871delG	p.Val1291Tyrfs*18	Novel	N/R	Deletion	Frameshift	PVS1, PM1, PM2, PP3	P	29
F83	c.4816_4817insGG	p.Val1606Glyfs*4,	Novel	N/R	Insertion	Frameshift	PVS1, PM1, PM2, PP3	P	36
F84	c.5107C>T	p.Gln1703*(%15)	CM143429	R(P)	Substitution	Non-sense	PVS1, PM1, PM2, PP3	P	36
F86	c.5827delG	p.Asp1943Metfs*15	Novel	N/R	Deletion	Frameshift	PVS1, PM1, PM2, PP3	P	39

LP: Likely pathogenic, P: Pathogenic, VUS: Variant of uncertain significance, R: Reported N/R: Not reported

Our results did not reveal any clear relationships between specific NF1 variants and phenotypes. Furthermore, no complete well-known genotype-phenotype correlation has been reported in the literature to date (12,18-20). Only three clear correlations of clinical significance have been identified in particular pathogenic NF1 variants. *NF1* whole-gene deletions are related with early-onset presentation of cutaneous neurofibromas, cognitive abnormalities, somatic overgrowth, and dysmorphic facial features (21,22). The c.2970-2972delAAT variant does not cause cutaneous or surface plexiform neurofibromas (23). Any of the c.5425C>T or c.5425C>A or c.5425C>G variants are related with café-au-lait spots, learning disabilities, short stature, and pulmonic stenosis but not cutaneous neurofibromas (24,25). However, C16 (13 years of age) with the c.2970-2972del variant had neurofibromas, which is a late-onset feature of NF1.

A novel c.1637dupT variation was detected in C85 (20 years of age) who had isolated pilocytic astrocytoma without cutaneous findings. The case of C11 with the c.1721+3A>C variation only had clinical signs of neurofibromas without the accompanying café-au-lait spots. Ocular manifestations such as Lisch nodules and optic nerve glioma were determined in 23% and 6.8% of all cases, which are lower frequencies in comparison with the literature (26,27). Additionally, the frequency of neurofibromas were lower than in the literature.

Mosaic NF1 variants cause mild forms of the NF1 phenotype (28). We detected two different variations with low variation fraction in C87 (c.3721C>T) and C90 (c.5107C>T). Sanger confirmations of these variants were consistent with NGS data. However, a molecular analysis of a second tissue could not be performed in these patients. Both of these patients had classical NF1 symptoms without family history and segmental involvement. We classified both of these patients as mosaic generalized NF1.

Conclusion

In conclusion, *NF1* genetic analysis was a supporting tool for the atypical presentation of NF1 cases especially in the prepubertal period. Additionally, genetic analysis before pregnancy provides preimplantation and prenatal genetic diagnosis for families with *NF1*.

Ethics

Ethics Committee Approval: The study was approved by the Ethical Committee of the Gaziantep University Medical Faculty (approval number: 65587614-774.99-291, date: 04/10/2017).

Informed Consent: Peripheral blood samples were obtained after taking informed consents from all participants or legal guardians of children under the age of 18.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.K., Design: E.K., Data Collection or Processing: H.M.A., E.K., Analysis or Interpretation: H.M.A., E.K., Literature Search: H.M.A., E.K., Writing: H.M.A., E.K.

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