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Detection of Novel NF1 Variants with Next-Generation DNA Sequencing Technology and Genotype-Phenotype Characteristics of Neurofibromatosis

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ABSTRACT

Objective: Neurofibromatosis type 1 (NF1, #162200) is a common neurological disorder with *de novo* or inherited germline mutations of the Neurofibromin (NF1, *613113). The purpose of this study is to increase the limited knowledge of NF1 in a small population-based dataset.

Materials and Methods: This study enrolled patients with clinically suspected NF1 referred to the Kayseri Training and Research Hospital, Medical Genetics Department, between 2015 and 2017. The local ethics committee approved this study. Next-generation sequencing was performed for the genetic analysis. The genetic, demographic, and clinical features of the participants were characterized.

Results: A total of 79 cases of NF1 were included. Of these cases, 40 were male, and 39 were female. The mean age was 11.9 years, and most were younger than 18 years. The most common complaint was café au lait macules. The 61 (77.3%) patients had pathogenic variants, and 16 (26.2%) were novel. Mostly affected mutation sites were exonic regions (n=54, 88.5%). The most common mutated exon was exon 38 (n=7, 11.5%), and most of the detected mutations were nonsense mutations (31%).

Conclusion: It is one of Türkiye's largest NF1 study groups, where all exons of the NF1 gene were analyzed. This study contributes novel variants to the literature. There was no mutational hotspot region, and no significant relationship between genotype and phenotype was observed. Further studies and large sample sizes are required to better understand the relationship between NF and genetic changes.

Keywords: NF1, NGS, novel variants, sequencing, Türkiye

INTRODUCTION

Neurofibromatosis Type 1 (NF1, #162200), also known as "Von Recklinghausen's Disease," is a common neurocutaneous disorder. It has an autosomal dominant pattern of inheritance with full penetrance. The reported incidence is 1/2600–1/4500 live births (1). Patients have mutational, allelic, or phenotypic heterogeneity (2). Some clinical manifestations are age-related (3, 4). The most common signs and symptoms of the disease are café au lait macules (CAL), Lisch nodules, axillary freckling, and multiple neurofibromas (5). The National Institute of Health (NIH) formulated these features with the Neurofibromatosis Conference Statement in 1988. The clinical diagnosis of the disease is based on the presence of two or more NIH criteria. In recent years, Karaconji et al. (6) described additional nondiagnostic cutaneous and extracutaneous signs when evaluating patients with NF1.

De novo or inherited germline mutations of the NF1 (*613113) gene cause the NF1 syndrome. NF1 is a tumor suppressor gene, located on the long arm of chromosome 17, and encodes the neurofibromin protein (7). Almost all tissues express the NF1 gene, but it is mainly expressed in the nervous system (2). Neurofibromin is a member of GTPase activating proteins and comprises 2018 amino acids (7). Its role is to impress multiple signaling pathways that convert active GTP-RAS to inactive GDP-RAS form. Consequently, it acts as a downregulator of cell growth and proliferation (2, 6, 7). Hence, any loss-of-function mutation of NF1 results in uncontrolled growth and increased cellular proliferation. Moreover, increased active RAS-GTP levels protect cells from apoptosis through the active PI3K/AKT/mTOR signaling pathway (7). Therefore, neurofibromin inactivation causes RAS hyperactivation and contributes to tumor formation (8, 9). Loss or mutations of the NF1 gene are an important step in NF1 tumorigenesis. Over 2000 mutations are present in the Human Gene Mutation Database (HGMD), and most are *de novo* mutations (3). The large size of the NF1 gene, pseudogenes, and the absence of a specific mutation and mutation site make genetic analysis challenging. Today, next-generation sequencing (NGS) is a practical and powerful tool for the detection of mutations. In the present study, we set out to increase the limited knowledge of NF1 in a small population-based dataset. We examined 79 patients with suspected NF1 with their genetic and clinical findings. We aimed to determine the distributions of NF1 variations and their relationship with clinical symptoms. Furthermore, our secondary goals were to identify a mutational hotspot and explore potential founder mutations of Türkiye. The findings of the study will contribute to a better knowledge of NF1 disease. It is also the largest study group in Türkiye, where all exons of the NF1 gene are analyzed.

MATERIALS and METHODS

In this study, patients diagnosed with NF1 in Kayseri Training and Research Hospital between 2015 and 2017 were retrospectively evaluated. The diagnosis was made in the presence of at least two NIH criteria. CAL-positive patients under the age of puberty (<12 years), although they did not meet the NIH criteria, were included in the study. Patients over the age of 12 years who did not meet the NIH criteria were excluded from the study. All participants were from the Central Anatolia Region of Türkiye. The files of patients were examined. The data were analyzed simultaneously with the examination period of the patients. NGS analysis results of the NF1 gene were noted. The genetic, demographic, and clinical features of the participants were characterized. The genetic results of some parents were also reached. For genetic analysis, genomic DNA was extracted with the DNA isolation kit (Zinexts Life Science Corporations, Taiwan) from peripheral blood samples. NGS was conducted using the NEXTflex Neurofibromatosis Amplicon Panel (NEXTflex Neurofibromatosis Amplicon Panel, BIOO Scientific Corp., USA). MiSeq NGS system (Illumina, USA) was utilized for the sequencing of the NF1 gene (RefSeq transcript NM_001042492.2). All the coding exons and exon–intron boundaries of the NF1 gene are covered. Information regarding enrichment performance and target coverage was obtained using the software SEQ (https://seq.genomize.com/) and Integrative Genomics Viewer (http://software.broadinstitute.org/software/igv/). The variant interpretation was made based on the American College of Medical Genetics and Genomics (ACMG 2015) practice guidelines. Data were collected from the dbSNP, EXAC, 1000G, ClinVar, and HGMD databases.

The Erciyes University Clinical Research Ethics Committee approved the present study (2017/282). At the time of enrollment, all patients, and/or parents provided written informed consent. This study has been conducted based on the Declaration of Helsinki.

Statistical Analysis

To analyze the data, IBM Statistics V25 package program (IBM Corp., Armonk, NY, USA) was employed. Descriptive statistics (number, percentage, and arithmetic mean) were used to describe the demographic characteristics, clinical features, and distribution of NF1 variations.

RESULTS

Seventy-nine patients were included in the study. Thirty-nine (49.4%) of them were female, and 40 (50.6%) were male. The mean age of patients at the time of evaluation was 11.9 years. Most were younger than 18 years (83.5%). The median age was 9 years, and the standard deviation was 12 years.

CAL was the main clinical symptom and was present in all patients. Other common symptoms were Lisch nodules (50/79, 63.3%), axillary or inguinal freckling (37/79, 46.8%), neurofibroma (20/79, 25.3%), skeletal manifestations (8/79, 10.1%), and hypertension (3/79, 3.8%). None of the patients had optic glioma. Of the 5.1% of patients (4/79) had neurofibroma as plexiform neurofibroma. Malignancy was present in 17.7% (n=14) of patients.

Table 1. Distribution of the clinical features of the study group (n=79)

Clinical features	Number of patients	
	n	%
CAL's	79	100
Iris Lisch nodules	50	63.3
Cutaneous neurofibroma	20	25.3
Plexiform neurofibroma	4	5.1
Freckling	37	46.8
Brain tumor	14	17.7
Bone lesion (scoliosis, short stature, pectus excavatus, etc.)	8	10.1
Hypertension	3	3.8
NIH criteria positive	59	74.6
Family history (NIH criteria <2)	52	65.8

CAL: café au lait macules; NIH: The National Institute of Health; 49 (62%) patients were under 12 years old; Male/Female = 40/39

Two-thirds of patients met (59/79, 74.6%) the NIH criteria. All patients who did not meet the NIH criteria (n=20, 25.49%) were under 12 years old. Table 1 provides the clinical features of patients.

Sixty-one of the 79 patients (77.3%) screened by the NGS method had the NF1 variation (Appendix 1). Of them, 16 (26.2%) cases were novel and not defined in the literature and were all heterozygous variants. The most affected mutation sites were exonic regions (n=54, 88.5%), and the most common mutated exon was exon 38 (n=7, 11.5%). Exon 38 was followed by exon 17 (n=4, 6.6%) and exon 21 (n=4, 6.6%), respectively. Of the detected variations, most were nonsense variants (31%). It was followed by missense variations, minor deletions, splice site changes, minor insertions, silent variations, and intronic region variations. Both deletions and insertions were variations that caused the frameshift. Most of the detected variants were pathogenic according to the ACMG criteria. The c.1924 C>T (n=3, 4.9%) and c.2446C>T (n=3, 4.9%) were the commonly detected nucleotide changes. No variation was found in the remaining 18 subjects.

In family history, 68.9% of parents had at least one NIH criterion such as CAL or Lisch nodules. However, a small amount of them was genetically analyzed (n=39, 49.4%). No variations were observed in 17.9% (n=7) of the families, as in their children. Nonetheless, 38.4% (n=15) of the variations were familial, whereas 43.5% (n=17) were *de novo* variations.

DISCUSSION

Neurofibromatosis (#162200), the most common neurocutaneous disease, is caused by loss-of-function mutations of the Neurofibromin (NF1, *613113) gene. With 350 kbp of genomic DNA, it is the most common mutated gene in the human genome (2, 10, 11). Over 2800 mutations have been reported in the literature (2, 12, 13). Globally, DNA mutations in NF1 are responsible for 88%–97% of clinically diagnosed NF1 cases. In the studies

conducted in Türkiye, NF1 mutation rates have been reported as 57%, 88%, and 72.4% (14–16). Our result, 77.2%, was compatible with previous data. The diagnosis is easy in the presence of well-known clinical features. However, early diagnosis and genetic counseling can be difficult because of variable expression, pseudogenes, and the absence of hotspots. Clinical symptoms can vary within a family or at different life stages of the same patient. The genotype–phenotype correlations cannot be established in most cases (11, 13, 14, 17). The gene has the highest mutation rate with 1/10,000 alleles per generation. Approximately 50% of patients have *de novo* mutations, and most are novel (2, 13, 18). In the current study, 53.12% (n=17) of variations were *de novo*. However, only 49.4% (n=39) of all the parents had NF1 genetic analysis. If all of the families had the segregation analysis, *de novo* variants could be diagnosed more frequently. The results obtained from genetic studies of NF1 families will allow counseling of families, the phenotypic characterization of variants, and identifying hotspot regions over time. Therefore, clinicians should perform genetic studies of NF1 families whenever possible.

The NIH consensus criteria may be sufficient for diagnosis in most patients, but several patients do not meet all of these criteria (5, 19). In the literature, there are individuals without neurofibromatosis according to NIH diagnostic criteria but with pathogenic NF1 mutations (20). Some mutation-positive families with multiple spinal neurofibromas or minimal cutaneous symptoms were reported. They have no other diagnostic features. An individual with optic tract glioma and a child with encephalocraniocutaneous lipomatosis are other examples of patients with NF1 mutations without NIH diagnostic features. The association of NF1 mutations with unusual phenotypes in these individuals is not understood (20). In the present study, 79 patients had suggestive findings of NF1 disease. However, not all patients met the NIH consensus criteria (n=20). Despite inadequate NIH consensus criteria, 75% (n=15) of patients were mutation-positive, all of these patients, except one, were under 8 years of age. This may be related to the young age of the patients and/or the low expression of the disease. These patients should be examined and followed up periodically for long-term NF1 findings. Additionally, 25% (n=5) of patients were negative for both NIH criteria and mutation profile. These patients should be followed up periodically for the appearance of NF1 clinical findings and examined in terms of diseases in the differential diagnosis. Additionally, genes, and mutations that have not yet been identified should be considered. In the study of Origone et al. (21), only café au lait macules were present in eight young patients. However, two of them were positive for the NF1 mutation. Clinical findings may not be sufficient to diagnose neurofibromatosis in young patients and patients with insufficient phenotypic expression (10). Many cases cannot be diagnosed clinically before the age of 8 years (17). The clinical and radiological findings of neurofibromatosis are more prominent in the 8–18 age groups and above (10). In the current study, our data were concordant with the literature. The majority of our patients over the age of 8 and over (91.3%) met the NIH criteria. NIH criteria were positive in 59 (74.6%) of the patients in the study group. Although the NIH criteria were positive, genetic results were negative in 13 (22%) patients. The NGS method, performed in the study, covers all coding exons, and exon–intron boundaries. However, it cannot detect the genetic variants involved in the promoter and intron noncoding regions or large genomic rearrangements or epigenetic mechanisms. A multistep mutation detection protocol could identify 95% of pathogenic NF1 mutations in individuals fulfilling the NIH diagnostic criteria (20). Therefore, research should be conducted with methods other than NGS, including multiplex ligation probe amplification (MLPA) in the patients, and mosaicism should be considered. Additionally, cDNA sequencing is recommended instead of DNA sequencing for NF1 sequence analysis (13).

Although there is no definitive genotype–phenotype relationship, several reported correlations exist. Truncating/splicing mutations in NF1 patients often have an earlier onset and pronounced clinical picture (10). Large (~1.4 Mb) genomic microdeletions covering the entire NF1 gene locus and adjacent genes show a severe clinical phenotype. Total deletion of the NF1 gene has been associated with dysmorphic facial features, severe developmental abnormalities, and early appearance of cutaneous neurofibromas (2, 20). A few cutaneous, subcutaneous, or plexiform neurofibromas were reported in exon 17 c.2970–2972 del AAT and have a milder phenotype than the complete NF1 gene deletions. Missense mutations of codon 844–848, which had a severe clinic, were associated with neurofibromas, optic pathway gliomas, malignant neoplasms, and skeletal abnormalities (2, 20). In our study, although we did not detect the complete NF1 gene deletion or codon 844–848 mutations, the c.2970–2972 del AAT deletion was detected in one patient with a Lisch nodule. The most common mutation in our study was the c.2446C>T change (n=3). In these patients, the clinic was mild, and skin findings (CALs, freckling, and neurofibromas) and Lisch nodules were prominent. Moreover, a milder form of the disease, characterized by the presence of only CALs and freckles, with changes in the amino acid found in p.Arg1809, has been reported in the literature (13). CAL is the most common feature in NF1. Nevertheless, it can be seen in the healthy population (11%–25%) (14). Familial multiple café au lait macules (Legius syndrome) in infancy and early childhood may be confused with the diagnosis of NF1. The absence of other findings of NF1 and having a family history of multiple CAL macules without other findings are important in terms of differential diagnosis (22). Due to the different phenotypes and molecular genetics of neurofibromatosis, cases with only CAL spots and/or neurofibromas are now considered neurofibromatosis. Kaçar et al. (14) 2021 recommended that patients with skin manifestations should be followed up carefully for the appearance of new features of the disease. Recently, Koczkowska et al. (11) reported a new genotype–phenotype correlation in which the pathogenic NF1 p.Met1149, p.Arg1276, or p.Lys1423 missense variants had an association with a Noonan-like phenotype.

In the current study, we screened all the coding exons and exon–intron boundaries of the NF1 gene with NGS analysis. The detected variations were nonsense (n=19, 31.2%), missense (n=17, 27.8%), deletions (n=12, 19.7%), insertions (n=5, 8.1%), silent (n=1, 1.7%), intronic (n=1, 1.7%), and splice site mutations (n=6, 9.8%) (Appendix 1). Compared with the HGMD database, the studied Turkish study group showed a significantly higher frequency of missense/nonsense mutations (59% vs. 28.1%) and a lower frequency of minor deletions (12% vs. 27.5%) (13). In the study of Kaçar et al. (14), nonsense variants were the most common mutation types. Kang et al. (10) identified the most common mutations

in their study group as frameshift followed by nonsense mutations. In the current study, nonsense mutations were the most common variation type. This difference may be due to the variability in the mutation types in the ethnicity/ancestry. Exon 21 is the largest exon of the NF1 gene (12). However, we found most of the variations in exon 38. NF1 mutations are dispersed equally throughout the gene (10, 18). Nevertheless, various NF1 regions have been examined, given that some exons have higher mutation density and recurrent mutations (18). In this context, Terzi et al. (18) analyzed exons 4, 16, 29, 31, and 37 of the gene in 100 Turkish NF1 patients. They identified two different mutations in exon 4 (c.496delGT and c.499delTGTT) and one novel mutation in exon 31 (c.5866delA). However, they could not identify recurrent founder mutations for rapid screening of patients. They reported that different populations have different hotspot regions and mutations. They suggested examining the entire gene to detect founder mutations of population groups. However, although we examined the entire gene as recommended, we could not obtain results pointing to a hotspot region for mutations, and it is necessary to work with larger study groups to find a mutational hotspot region in the genetic analysis of NF1. Additionally, we have demonstrated that sequence analysis is not sufficient to examine the entire NF1 gene, and additional methods are essential, especially MLPA, in the genetic analysis of NF1.

CONCLUSION

In conclusion, this is the largest study group in Türkiye, where all exons of the NF1 gene are analyzed. Cases with suspected NF1 should be investigated carefully and followed up clinically. There is no relationship between genotype and phenotype, similar to previous studies. Identification of the genetic causes of NF1 disease has great diagnostic utility, as it can confirm the etiology of the disease in the presence of inadequate clinical findings. The results of this study enhance our knowledge of the NF1 mutation profile and distribution in patients. Sequence analysis is not sufficient alone to examine the entire NF1 gene. MLPA should be performed, considering the possibility of large deletions and duplications. Moreover, research on larger study groups and long-term follow-up of patients will provide beneficial results.

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Informed Consent: Written informed consent was obtained from patients who participated in this study.

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Appendix 1. Mutational data of patients with NF1 (NM_001042492.3; boldfaced lettering indicate novel variants) and NF1 variant information

	Coding	Amino acid change	Variant effect	Location	RS numbers	ClinVar	ACMG (2015) classification	De novo/ inherited
CS 01	None	None	None	None	None	None	None	None
CS 02	**c. 5321_5322 ins G**	**p.Q1775A fs*7**	Deletion	E 38	–	–	P	De novo
CS 03	**c. 7328_7329 ins TA**	**p.T2444I fs*13**	Insertion	E 50	–	–	P	De novo
CS 04	c.1198C>T	p.Q400*	Nonsense	E 11	rs1597682751	P	P	Inherited
CS 05	c.1198 C>T	p.Q400*	Nonsense	E 11	rs1597682751	P	P	Inherited
CS 06	c.6819 G>A	p.K2273K	Silent	E 46	rs1060500373	VUS	LP	De novo
CS 07	**c.1707_1708 ins A**	**p.F570I fs*5**	Insertion	E 15	–	–	P	De novo
CS 08	None	None	None	None	None	None	None	None
CS 09	c.7549 C>T	p.R2517*	Nonsense	E 51	rs866445127	P	P	–
CS 10	c.3445A>G	p.M1149V	Missense	E 26	rs1187097568	P	P	–
CS 11	c.3445A>G	p.M1149V	Missense	E 26	rs1187097568	P	LP	–
CS 12	c.5609G>A	p.R1870Q	Missense	E 38	rs786202112	P	P	–
CS 13	c.3104T>A	P.M1035K	Missense	E 23	rs137854553	LP	LP	–
CS 14	c.4600 C>T	p.R1534 *	Missense	E 35	rs760703505	P	P	De novo
CS 15	c.484 C>T	p.Q162*	Nonsense	E 5	rs1555607073	P	P	De novo
CS 16	c.2446 C>T	p.R816*	Nonsense	E 21	rs886041347	P	P	–
CS 17	c.1174C>T	p.Q392*	Nonsense	E 10	rs1597682137	P	P	–
CS 18	c.5305C>T	p.R1769*	Nonsense	E 38	rs876657714	P	P	–
CS 19	**c.4557_4561delGCAGT**	**p.G1520S fs*8**	Deletion	E 34	–	–	P	De novo
CS 20	c.7909C>T	p.R2637*	Nonsense	E 54	rs786201367	P	P	De novo
CS 21	c.1924 C>T	p.Q642*	Nonsense	E 17	–	–	P	Inherited
CS 22	None	None	None	None	None	None	None	None
CS 23	c.1924 C>T	p.Q642*	Nonsense	E 17	–	–	P	Inherited
CS 24	c.1924 C>T	p.Q642*	Nonsense	E 17	–	–	P	Inherited
CS 25	c.205-1G>C	–	Splicing	I 2	rs1555605362	P	P	Inherited
CS 26	c.205-1G>C	–	Splicing	I 2	rs1555605362	P	P	Inherited
CS 27								
CS 28	c.1527+1G>T	–	Splicing	I 13	rs1060500331	P	P	De novo
CS 29	**c.7348 C>T**	**p.R2450***	Nonsense	E 50	rs786202457	P	P	De novo
CS 30	c.5305 C>T	p.R1769*	Nonsense	E 38	rs876657714	P	P	Inherited
CS 31	c.2446 C>T	p.R816*	Nonsense	E 21	rs886041347	P	P	De novo
CS 32	**c.495_498delTGTT**	p.C167Qfs*10	Deletion	E 5	rs786201874	P	P	De novo
CS 33	**c.495_498delTGTT**	p.C167Qfs*10	Deletion	E 5	rs786201874	P	P	De novo
CS 34	**c.3212 C>T**	**p.A1071V**	Missense	E 25	–	LP	LP	Inherited
CS 35	**c.104_105delGT**	**p.S35Nfs*2**	Deletion	E 2	–	–	P	De novo
CS 36	c.1466A>G	p.Y489C	Missense	E 13	rs137854557	p	P	De novo
CS 37	**c.5294 C>G /c.6951 G>A**	**p.T1765S/p.W2317***	Missense	E 38 E 47	–		LP/P	Inherited
CS 38	**c.5294 C>G /c.6951 G>A**	**p.T1765S/p.W2317***	Missense	E 38 E 47	–		LP/P	Inherited
CS 39	**c.479+36 A>G**	–	Intronic	I 4	–		LP	Inherited
CS 40	None	None	None	None	None	None	None	None
CS 41	**c.1542delG**	**p.E517K fs* 9**	Deletion	E 14	–		LP	De novo
CS 42	c.6818 A>G	p.K2273R	Missense	E 45	rs1060500344	Conflicting	VUS	–
CS 44	c.4330 A>G	p.K1444E	Missense	E 32	rs137854550	P	P	–
CS 45	c.245C>T	p.S82F	Missense	E 3	rs199474729	Conflicting	P	–
CS 46	c.4835+1G>A	–	Splicing	I 36	rs1085307819	P	P	–

Appendix 1 (cont). Mutational data of patients with NF1 (NM_001042492.3; boldfaced lettering indicate novel variants) and NF1 variant information

	Coding	Amino acid change	Variant effect	Location	RS numbers	ClinVar	ACMG (2015) classification	De novo/ inherited
CS 47	c.978delA	p.K326Nfs*50	Deletion	E 9	rs1085307819	P	P	–
CS 48	c.2990 G>A	p.R997K	Missense	E 22	rs1555614462	P	P	Inherited
CS 49	c.2990 G>A	p.R997K	Missense	E 22	rs1555614462	P	P	Inherited
CS 50	c.2446 C>T	p.R816*	Nonsense	E 21	rs886041347	P	P	–
CS 51	c.2970_2972 del AAT	p.M992del	Deletion	E 22	rs267606606	P	P	–
CS 52	None	None	None	None	None	None	None	None
CS 53	c.5609+5G>T	–	Splicing	I 38	rs1597832498	Conflicting	LP	De novo
CS 54	**c.7591 C>T**	**p.Q2531***	Nonsense	E 51	rs1555536372	P	P	–
CS 56	c.888+1 G>A	–	Splicing	I 8	rs1135402799	P	P	–
CS 57	**c.4871_4872insAA**	**p.Y1625Nfs*6**	Insertion	E 37	–		P	–
CS 58	None	None	None	None	None	None	None	None
CS 59	**c.1733delT**	**p.Y580Tfs*6**	Deletion	E 16	–		LP	–
CS 60	**c.6212delA**	**p.Q2071Hfs*11**	Deletion	E 42	–		LP	–
CS 61	c.5588G>A	p.G1863D	Missense	E 38	rs1597832460	VUS	VUS	–
CS 62	**c.2486_2487 insT**	**p.D830***	Insertion	E 21	–		P	Inherited
CS 63	c.2230 G>A	p.W777*	Nonsense	E 20	rs1555613983	P	P	De novo
CS 64	None	None	None	None	None	None	None	None
CS 65	c.625 C>T	p.Q209*	Nonsense	E 6	rs786203448	P	P	–
CS 66	c.1885 G>A	p.G629R	Missense	E 17	rs199474738	P	P	–
CS 67	None	None	None	None	None	None	None	None
CS 68	None	None	None	None	None	None	None	None
CS 69	**c.7140_7141 insA**	**p.N2381Kfs*5**	Insertion	E 48	–		LP	–
CS 70	c.5902C>T	p.R1968*	Nonsense	E 40	rs137854552	P	P	–
CS 71	c.1397 del T	**p.T467Hfs*6**	Deletion	E 13	–		P	–
CS 72	c.3055 G>A	p.V1019I	Missense	E 23	rs1567849826	VUS	P	–
CS73	c.1754_1757delTAAC	p.T586Lfs*18	Deletion	E16	rs786202782	P	P	–
CS 74	None	None	None	None	None	None	None	None
CS 75	None	None	None	None	None	None	None	None
CS 76	None	None	None	None	None	None	None	None
CS 77	None	None	None	None	None	None	None	None
CS 78	None	None	None	None	None	None	None	None
CS 79	None	None	None	None	None	None	None	None

*P: Pathogenic; LP: Likely pathogenic; VUS: Variant of unsignificant; E: Exonic; I: Intronic; CS: Current study; NF1: Neurofibromatosis type 1.