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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

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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, Türkive

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 (<12years), 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). MiSeg 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://seg.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 exonintron 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.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – AK; Design – AK, HG, HP; Supervision – AK, HP, HG; Resource – HP, HG; Materials – ASG, SK, NB, AS, SFÇ; Data Collection and/or Processing – AK, BB, ME; Analysis and/or Interpretation – AK, ZFK; Literature Search – AK, SYÖ; Writing – AK; Critical Reviews – ÜGÖG,HP.

Conflict of Interest: The authors have no conflict of interest to declare.

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Image <thimage< th="">ImageImageImage<t< th=""><th>Append</th><th colspan="8">Appendix 1. Mutational data of patients with NF1 (NM_001042492.3; boldfaced lettering indicate novel variants) and NF1 variant information</th></t<></thimage<>	Append	Appendix 1. Mutational data of patients with NF1 (NM_001042492.3; boldfaced lettering indicate novel variants) and NF1 variant information							
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CS 04c1198c1940°NorsensF.1r159768275P.P.P.InheritedCS 05c109 C>Tp.4223°CNorsensF.10r105050275P.P.P.DenovoCS 05c170_179 Ins Ap.F270 fs*5InserioF.15NoreNoreNoreCS 05c749p.P251°CNore <td>CS 02</td> <td>c. 5321_5322 ins G</td> <td>p.Q1775A fs*7</td> <td>Deletion</td> <td>E 38</td> <td>-</td> <td>-</td> <td>Р</td> <td>De novo</td>	CS 02	c. 5321_5322 ins G	p.Q1775A fs*7	Deletion	E 38	-	-	Р	De novo
CS 00 c198 C-T pQ400° Nonese F1 n57675757 P P P P P CS 00 c1707 J78 ins A pF570 fr5 None F P P P P CS 00 c7307 L78 ins A P P570 fr5 None F P P P P CS 00 c7349 CA P P P P P P CS 01 c7349 CA pA1149V Nonese F2 r181707576 P P P CS 11 c3445A-G pA1149V Missee E3 r181707576 P P P CS 12 c3609G-A pA1149V Missee E3 r181707576 P P P CS 13 c3445A-G PA1130A Missee E3 r18760573 P P P CS 14 c446C+T pA1620 Nonese E1 r18560137 P P P CS 14 c345C+T pA1620 Nonese E1 r38560137 P P P CS 14 c345C+T pA1620 Nonese E1 r38560137 P P P CS 24 c340C+T <	CS 03	c. 7328_7329 ins TA	p.T2444I fs*13	Insertion	E 50	-	-	Р	De novo
CS 00cis09 GAApiR2073KSilentF460silentomF170Fa70F180 <th< td=""><td>CS 04</td><td>c.1198C>T</td><td>p.Q400*</td><td>Nonsense</td><td>E 11</td><td>rs1597682751</td><td>Р</td><td>Р</td><td>Inherited</td></th<>	CS 04	c.1198C>T	p.Q400*	Nonsense	E 11	rs1597682751	Р	Р	Inherited
CS 09c.1707_1708 in Ap.F570 fa*5insertionF15PDenoxoCS 08NoneNoneNoneNoneNoneNoneNoneNoneNoneNoneCS 00c.7549 C>TB2517*NonesE10R\$6645127PP-CS 11c.3445A-5Gp.M1149VMissensE26r.1187097568PP-CS 12c.5609C>Ap.M1149VMissensE26r.1187097583LPP-CS 13c.5609C>Ap.R1870QMissensE38r.876073135LPPDenovCS 14c.4600 C>Tp.R1534*MissensE3r.87565714PDenovDenovCS 15c.444 C>Tp.R154*NonsensE10r.855607073PPDenovCS 16c.444 C>Tp.R159*NonsensE10r.855607137PPDenovCS 16c.444 C>Tp.R167*NonsensE10r.85607147PDDenovCS 15c.45574561deGAGMp.G1620*NonsensE14r.85601367PDDenovCS 2c.1942 C>Tp.G642*NonsensE17NoneNoneCS 2c.1942 C>Tp.G642*NonsensE17NoneNoneCS 2c.1942 C>Tp.G642*NonsensE17NoneNoneCS 2c.1942 C>Tp.G642*NonsensE17<	CS 05	c.1198 C>T	p.Q400*	Nonsense	E 11	rs1597682751	Р	Р	Inherited
CS 08NomeN	CS 06	c.6819 G>A	p.K2273K	Silent	E 46	rs1060500373	VUS	LP	De novo
CS 09c.7549 C>Tp.R2517*NonsenseF.1ns66445127PP-CS 10c.3445A>Gp.M1149VMissenseE.26n51187097568PP-CS 11c.3445A>Gp.M1149VMissenseE.36n5187097568PP-CS 12c.5609CAp.R1870CMissenseE.38n5786202112PP-CS 13c.3404T>AP.M1035KMissenseE.35n57657035PPDe novoCS 14c.4600C>Tp.R1524NonsenseE.10n57657037PDe novoCS 15c.484 C>Tp.Q392*NonsenseE.10n57562137PPDe novoCS 16c.4357.4601deGCAGTp.G15205 6*8DeltonE.34PDe novoCS 21c.4357.4601deGCAGTp.G15205 6*8DeltonE.34r57662137PPDe novoCS 22A09C>Tp.G427NonsenseE.14r57662137PPDe novoCS 23c.1924 C>Tp.G427NonsenseE.14r57650136PPDe novoCS 24c.1924 C>Tp.Q642*NonsenseE.17r576505362PPDe novoCS 34c.1924 C>Tp.Q642*NonsenseE.17r515505362PPDe novoCS 4c.1924 C>Tp.Q642*NonsenseE.14r515605362PPDe novoCS 4c.1924 C>Tp.Q642*NonsenseE	CS 07	c.1707_1708 ins A	p.F570I fs*5	Insertion	E 15	-	-	Р	De novo
CS 10c.3445A-Gp.M1149VMasonsE.6r.1187097568P.P.P.P.CS 11c.3609CAp.M1149VMasonsE.30r.318709756P.P.P.P.CS 12c.3609CAp.R1870QMasonsE.30r.3786201712P.P.P.P.CS 13c.3104TAp.R1035KMasonsE.31r.3760703505P.P.DenovoCS 14c.4600 C>Tp.Q162'NonsensE.10r.585040137P.P.DenovoCS 15c.484 C>Tp.Q162'NonsensE.10r.58504137P.P.P.P.CS 16c.2446 C>Tp.R150*NonsensE.10r.58504137P.P.P.P.CS 17c.174C>Tp.Q392'NonsensE.12r.58604137P.P.P.P.P.CS 18c.305C>Tp.Q162'NonsensE.17P.P.NonsenP.<	CS 08	None	None	None	None	None	None	None	None
CS 11c.3445Ap.M149VMisanseE.26m.N1870QMisanseE.28m.N28620211P.0P.0-CS 13c.3104T>AP.M135VMisanseE.38m.738545C3P.0P.0-CS 13c.3400CSTp.M153VMisanseE.35m.7507305P.0P.0DenovoCS 15c.4600CSTp.R153VMisanseE.5m.755560707P.0P.0DenovoCS 16c.2446 CSTp.R164'NonsenseE.10m.857669714P.0P.0-CS 17c.1174CSTp.Q392NonsenseE.30m.87669714P.0P.0-CS 18c.3505CSTp.R169'NonsenseE.34m.87669714P.0P.0-CS 10c.1924CSTp.R2637'NonsenseE.34m.87669714P.0P.0NonsenseCS 11c.1924CSTp.R2637'NonsenseE.17m.87669714P.0NonsenseNonsenseF.17-<	CS 09	c.7549 C>T	p.R2517*	Nonsense	E 51	rs866445127	Р	Р	-
CS 12c.5609S-Ap.R1870QMissenseE.38r.8786202112P.P.P.P.CS 14c.4600C>TP.M1035KMissenseE.23r.813785455I.P.I.P.P.DenovoCS 14c.484 C>Tp.R154*MissenseE.21r.85507073P.P.DenovoCS 15c.484 C>Tp.R16*NonsenseE.10r.85507073P.P.P.DenovoCS 16c.444 C>Tp.R16*NonsenseE.10r.85507073P.P.P.P.CS 16c.444 C>Tp.R16*NonsenseE.10r.85507073P.P.P.P.CS 16c.4345 C>Tp.R16*NonsenseE.10r.85507073P.P.P.P.CS 18c.4345 C>Tp.R16*NonsenseE.10r.85601737P.P.P.P.CS 21c.4557 L451 LGCAGp.R2637NonsenseE.17P.P.P.P.P.CS 22NoneNoneNonsenseE.17P.P.P.InheritedCS 23c.1924 C>Tp.Q642*NonsenseE.17P.P.P.InheritedCS 24c.1924 C>Tp.Q642*NonsenseE.17P.P.P.InheritedCS 25c.2051 G>CP.P.Q.642*NonsenseE.17P.P.P.NonsenseE.17P.P.P.NonsenseE.17P.P.Nonsense	CS 10	c.3445A>G	p.M1149V	Missense	E 26	rs1187097568	Р	Р	-
CS13c.104T>AP.N1035KMissensE.23r37874553J.P.LP-CS14c.4600 C>Tp.R1534*MissensE.35rs76070350P.0P.0DenovoCS15c.2446 C>Tp.Q162*NonsensE.31rs155670773P.0P.0-CS16c.2446 C>Tp.Q392*NonsensE.10rs1557682137P.0P.0-CS17c.1174C>Tp.Q392*NonsensE.38rs87665774P.0P.0-CS18c.3505C>Tp.R1769*NonsensE.38rs87665774P.0DenovoDenovoCS19c.4557456164CCCCp.R2637*NonsensE.44rs78520137P.0DenovoDenovoCS20c.7909C>Tp.Q642*NonsensE.17P.0InheritedCS23c.1924 C>Tp.Q642*NonsensE.17P.0InheritedCS24c.1924 C>Tp.Q642*NonsensE.17P.0InheritedCS25c.051-G>Cp.Q642*NonsensE.17s155505362P.0P.0InheritedCS24c.1924 C>Tp.Q642*NonsensE.17s155505362P.0P.0InheritedCS25c.051-G>Cp.R169*NonsensE.30rs75505362P.0P.0InheritedCS24c.1924 C>Tp.R169*NonsensE.30rs76507147P.0D.0D.0D.0CS36c.1527+1G>Tp	CS 11	c.3445A>G	p.M1149V	Missense	E 26	rs1187097568	Р	LP	-
CS14c4600 C>Tp,R1534*MissenseF.35rs76070350P.P.De novoCS15c.444 C>Tp,R164*NonsenseF.21rs185601137P.P.P.CS16c.1446 C>Tp,R316*NonsenseF.21rs86041347P.P.P.CS17c.1174C>Tp,Q32*NonsenseF.10rs87657114P.P.P.P.CS18c.3505C>Tp,R169*NonsenseF.34rs7650714P.P.P.P.CS19c.4557.4561deGCAGTp,G15205.65*DeletionF.34rs78621737P.P.P.P.P.CS21c.1992.C>Tp,C642*NonsenseF.17P.<	CS 12	c.5609G>A	p.R1870Q	Missense	E 38	rs786202112	Р	Р	-
C 15c.484 C>Tp.Q162°NonsenseE 1rs155660703P.P.DenovoCS 16c.2446 C>Tp.R316°NonsenseE 1rs886041347P.PCS 17c.1174C>Tp.Q392°NonsenseE 10rs1597665713P.P.PCS 18c.3505C>Tp.R169°NonsenseE 30rs87665714P.P.DenovoCS 19c.4557_456146CAGTp.G15205 fr.8DeletionE 34P.DenovoCS 20c.7909C>Tp.R637°NonsenseE 17-P.P.NoneroCS 21c.1924 C>Tp.Q642°NonsenseE 17-P.NoneNoneCS 23c.1924 C>Tp.Q642°NonsenseE 17-P.NoneroNoneroCS 24c.1924 C>Tp.Q642°NonsenseE 17P.InheritedCS 25c.205-IG>C-SplicingI 2rs155560532P.P.InheritedCS 26c.205-IG>C-SplicingI 2rs155560532P.P.NoneroCS 28c.1527+IG>TSplicingI 3rs16505143P.P.P.P.CS 29c.5305 C>Tp.R16704°NoneseE 10rs16505324P.P.D.D.CS 30c.1527+IG>Tp.R16704°NoneseE 20rs7862137P.P.D.D.CS 30c.1524-IGST </td <td>CS 13</td> <td>c.3104T>A</td> <td>P.M1035K</td> <td>Missense</td> <td>E 23</td> <td>rs137854553</td> <td>LP</td> <td>LP</td> <td>-</td>	CS 13	c.3104T>A	P.M1035K	Missense	E 23	rs137854553	LP	LP	-
CS16c.2446 C>Tp.R816°NonsenseF.21rs88604137PPPPCS17c.1174C>Tp.Q392°NonsenseF.10rs57628213PPPCCS18c.3505C>Tp.R1769°NonsenseF.30rs7860714PPDDCS19c.7099C>Tp.G15205 fs78NonsenseF.44rs78621037PPDDDCS21c.7092C>Tp.Q642°NonsenseF.17PMonterNoneCS23c.1924 C>Tp.Q642°NonsenseF.17PMonterInheritedCS24c.1924 C>Tp.Q642°NonsenseF.17PMonterInheritedCS24c.205-1G>CSlicing12rs155605362PPInheritedCS25c.205-1G>CSlicing12rs155605362PPMonterInheritedCS24c.305-GTp.R169°Slicing12rs155605362PPDDDCS25c.305-GTp.R169°NonsenseF.50rs78602437PPDDDDCS3c.305-GTp.R169°NonsenseF.20rs7860217PPDDDDDDDDDDDDDDDDDDDDDDDDDD </td <td>CS 14</td> <td>c.4600 C>T</td> <td>p.R1534 *</td> <td>Missense</td> <td>E 35</td> <td>rs760703505</td> <td>Р</td> <td>Р</td> <td>De novo</td>	CS 14	c.4600 C>T	p.R1534 *	Missense	E 35	rs760703505	Р	Р	De novo
CS17c.114C>Tp.Q392NorsensE.10r.15708213P.P.P.P.CS18c.3305C>Tp.R1769NorsensE.38r.87665714P.P.P.CS19c.4557_4561delCACHp.G152054*8DeleionF.34r.87661731P.P.DenoroCS20c.7090C>Tp.R2637*0NorsensE.17r.8762133P.P.NoreNoreCS21c.1924C>Tp.Q642*0NorsensE.17-0P.NoreNoreCS23c.1924C>Tp.Q642*0NorsensE.17-1P.P.NoreCS24c.1924C>Tp.Q642*0NorsensE.17-1P.P.NoreCS25c.2054C>Tp.Q642*0NorsensE.17-1P.P.NoreCS24c.1924C>Tp.Q642*0NorsensE.17-1P.P.NoreCS25c.2054C>Tp.Q642*0NorsensE.17-1P.P.NoreCS26c.2054C>Tp.Q642*0NorsensE.17-1P.P.NoreCS26c.2054C>Tp.Q642*0NorsensE.17s.15556536P.P.NoreCS26c.2054C>Tp.R4450*0NorsensE.16r.55565554P.P.NoreNoreCS26c.5274C>Tp.R450*0NorsensE.50r.55650542P.P.NoreNoreCS26c.5274C>Tp.R450*0NorsensE.5	CS 15	c.484 C>T	p.Q162*	Nonsense	E 5	rs1555607073	Р	Р	De novo
CS18c3305C>TpR1769°NonsenseE 38rs876657714PP-CS19c4557_4561delGCAGTpG15205 fs*8DeletionE 34PDe novoCS20c7909C>Tp.R2637*NonsenseE 54rs786201367PPDe novoCS21c1924 C>Tp.Q642°NonsenseE 17PInheritedCS23c1924 C>Tp.Q642°NonsenseE 17PInheritedCS24c1924 C>Tp.Q642°NonsenseE 17PInheritedCS25c205-1G>C-Splicing12rs155505362PPInheritedCS26c205-1G>C-Splicing12rs155505362PPInheritedCS27c205-1G>C-Splicing12rs155605362PPDe novoCS28c1954 S>TSplicing12rs155605362PPDe novoCS28c305 C>Ts.P42450*Splicing12rs155605362PPDe novoCS29c338 SC>Ts.P174SplicingSplicing13rs16050731PDe novoCS29c346 S>Ts.P174SplicingSplicing13rs16050731PDe novoCS29c345 SC>Ts.P174SplicingSplicingF3rs16050731PDe novoCS30c305 C>Tp.R16951NonsenseE 3rs76	CS 16	c.2446 C>T	p.R816*	Nonsense	E 21	rs886041347	Р	Р	-
CS19c4557_4501deGCAMp.61520 Sr450DeltonP.44DeltonP.44DeltonP.44DeltonP.45 <th< td=""><td>CS 17</td><td>c.1174C>T</td><td>p.Q392*</td><td>Nonsense</td><td>E 10</td><td>rs1597682137</td><td>Р</td><td>Р</td><td>-</td></th<>	CS 17	c.1174C>T	p.Q392*	Nonsense	E 10	rs1597682137	Р	Р	-
CS20c.7909C×Tp.R2637*NonsenseE 54r578201377PPDe novoCS21c.1924 C>Tp.Q642*NonsenseE 17PInheritedCS23c.1924 C>Tp.Q642*NonsenseE 17PInheritedCS24c.1924 C>Tp.Q642*NonsenseE 17PInheritedCS25c.205-1G>C-Splicing12r55505362PPInheritedCS26c.205-1G>C-Splicing12r55505362PPInheritedCS27Splicing12r55505362PPInheritedCS28c.1527+1G>T-Splicing13rs105050313PPDe novoCS30c.5305 C>Tp.R2450*NonsenseE 50rs78620457PPDe novoCS31c.2446 C>Tp.R316*NonsenseE 21rs85061343PPDe novoCS33c.495_498deffGTTp.C1670fs*10DeltonE 51rs78621874PPDe novoCS33c.104_105defGTp.C1670fs*10DeltonE 13rs78621874PPDe novoCS34c.146A>Gp.117655/p.W231*MissenseE 31rs13785457PPDe novoCS33c.146A>Gp.117655/p.W231*MissenseE 31rs13785457PPDe novoCS34c.146A>Gp.117655/p.W231*Missense	CS 18	c.5305C>T	p.R1769*	Nonsense	E 38	rs876657714	Р	Р	-
CS21c.1924 C>Tp.Q642°NonesseF 17PInheritedCS22None <t< td=""><td>CS 19</td><td>c.4557_4561delGCAGT</td><td>p.G1520S fs*8</td><td>Deletion</td><td>E 34</td><td>-</td><td>-</td><td>Р</td><td>De novo</td></t<>	CS 19	c.4557_4561delGCAGT	p.G1520S fs*8	Deletion	E 34	-	-	Р	De novo
CS22NoneNo	CS 20	c.7909C>T	p.R2637*	Nonsense	E 54	rs786201367	Р	Р	De novo
CS23c.1924 C>Tp.Q642*NorsenseF.17NNInheritedCS24c.1924 C>Tp.Q642*NorsenseF.17rrPInheritedCS25c.205-IG>C-SplicingI2rs15556052PPInheritedCS26c.205-IG>C-SplicingI2rs155560532PPInheritedCS27SplicingI2rs15560503PPDInheritedCS28c.1527+IS>T-SplicingI3rs10605031PPDDDCS39c.1527+IS>Tp.R150*NonsensE30rs786202457PPDD <td>CS 21</td> <td>c.1924 C>T</td> <td>p.Q642*</td> <td>Nonsense</td> <td>E 17</td> <td>-</td> <td>-</td> <td>Р</td> <td>Inherited</td>	CS 21	c.1924 C>T	p.Q642*	Nonsense	E 17	-	-	Р	Inherited
CS24c.1924 C>Tp.Q642°NonsenseF.17NPInheritedCS25c.2051G>C-ASplicing12r.155560562PPInheritedCS26c.2051G>CSplicing12r.155560562PPInheritedCS27Splicing12r.155560563PPDInheritedCS28c.15271G>CSplicing13r.16050031PPDDCS30c.305 C>Tp.R2450*NonsensE 50r.87650714PPDDDCS31c.2446 C>Tp.R316*NonsensE 21r.886041347PPDDDDCS33c.305 C>Tp.R1679*NonsensE 21r.886041347PPDDD <td< td=""><td>CS 22</td><td>None</td><td>None</td><td>None</td><td>None</td><td>None</td><td>None</td><td>None</td><td>None</td></td<>	CS 22	None	None	None	None	None	None	None	None
CS 25c.205 florSplicing12rs15560532PPInheritedCS 26c.205 florSplicing12rs15560532PPInheritedCS 27Splicing13rs10605031PPDe novoCS 28c.1527 florSplicingF3rs76202457PPDe novoCS 29c.7348 C>Tp.R2450*NonsensE 30rs7660714PPDe novoCS 30c.2446 C>Tp.R1769*NonsensE 31rs86041347PPDe novoCS 31c.2446 C>Tp.R1670*DeletingE 51rs7820174PPDe novoCS 32c.495_498detIGTTp.C167Qfs*10DeletingE 51rs7820174PPDe novoCS 33c.495_498detIGTTp.G167Qfs*10DeletingE 52rs7820174PPDe novoCS 34c.495_498detIGTTp.G167Qfs*10DeletingE 53rs7820174PPDe novoCS 35c.104_105deIGTp.G167Qfs*10DeletingE 53rs7820174PPDe novoCS 35c.104_105deIGTp.S35Ns*2DeletingE 32rs7820174PDe novoCS 36c.104_105deIGTp.Y4980CMisenseE 32rs7378557PDe novoCS 36c.104_105deIGTp.T17657p.W231MisenseE 32rs7378557PDr/PDicherited </td <td></td> <td>c.1924 C>T</td> <td>p.Q642*</td> <td>Nonsense</td> <td>E 17</td> <td>-</td> <td>-</td> <td>Р</td> <td>Inherited</td>		c.1924 C>T	p.Q642*	Nonsense	E 17	-	-	Р	Inherited
CS 26c.205 f.G>C-Spline12rs155605.22PPInheritedCS 27Splicing1.3rs1060503.31PPDe novoCS 28c.1527+1G>TSplicing1.3rs1060503.31PPDe novoCS 29c.7348 C>Tp.R2450*NonsensE.50rs786202457PPDe novoCS 30c.5305 C>Tp.R1769*NonsensE.31rs786201374PPDe novoCS 31c.2446 C>Tp.R167NonsensE.12rs88041347PPDe novoCS 32c.495_498detTGTTp.C1670fs*10DeletionE.5rs78201874PPDe novoCS 33c.495_498detTGTTp.C1670fs*10DeletionE.5rs78201874PPDe novoCS 34c.495_498detTGTTp.C1670fs*10DeletionE.5rs78201874PPDe novoCS 34c.495_498detTGTTp.G1670fs*10DeletionE.5rs78201874PPDe novoCS 35c.495_498detTGTTp.41071VMisenseE.5rs78201874PPDe novoCS 35c.104_105deGTp.41071VMisenseE.5rs17385557PPDe novoCS 35c.146A>Gp.17655/p.W231*MisenseE.38rs173854557PP/PInheritedCS 35c.294C>G/c.6951AAp.17655/p.W231*MisenseE.38 F.4PInheritedInh	CS 24	c.1924 C>T	p.Q642*	Nonsense	E 17	-	-	Р	Inherited
CS 27CS 28c.1527+1G>T-Splicing1.3r106050031P.P.De novoCS 29c.7348 C>Tp.R2450*NonsenseE.50r376202457P.P.De novoCS 30c.2446 C>Tp.R1769*NonsenseE.38r87665714P.P.De novoCS 30c.2446 C>Tp.R167*NonsenseE.38r87665714P.P.De novoCS 31c.2446 C>Tp.R167*NonsenseE.38r3766201374P.P.De novoCS 32c.495_498detTGTTp.C167Qfs*10DeletinF.r3786201374P.P.De novoCS 33c.495_498detTGTTp.C167Qfs*10DeletinE.5r3786201374P.P.De novoCS 34c.495_498detTGTTp.C167Qfs*10DeletinE.5r3786201374P.P.De novoCS 35c.495_498detTGTp.41071VMissenseE.5r3785201374P.P.De novoCS 35c.104_105detGTp.35Nfs*2DeletinE.13r313785457p.P.De novoCS 36c.1466A>Gp.T1655/p.W2317MissenseE.38 E4PLP/PInderitedCS 37c.5294 C>G.c.6951 G>p.T1655/p.W2317MissenseE.38 E4P.LP/PInderitedCS 36c.5294 C>G.c.6951 G>p.T1655/p.W231MissenseE.38 E4P.LP/PInderitedCS 36c.5294 C>G.c.6951 G>p.E17T fs*9Deletin<	CS 25	c.205-1G>C	-	Splicing	I 2	rs1555605362	Р	Р	Inherited
CS 28c.1527+1G>T-RSplicing13rs10605033PPDenoveCS 29c.7348 C>Tp.R2450*NonsensE 50rs78620457PPDenoveCS 30c.305 C>Tp.R1769*NonsensE 38rs87665714PPDenoveCS 31c.2446 C>Tp.R167NonsensE 38rs86041347PPDenoveCS 32c.495_498detTGTp.R1670*10DeletinFrs78620187PPDenoveCS 33c.495_498detTGTp.C167Qfs*10DeletinE 5rs78620187PDenoveDenoveCS 34c.3212 C>Tp.A1071VMissensE 5rs78620187PDenoveDenoveCS 35c.104_105delGTp.S35Nfs*2DeletinE 2rs13785557p.R1DenoveDenoveCS 35c.164_6ASGp.Y489CMissensE 13rs137854557p.R1DenoveDenoveCS 36c.5294 C>G.6.9951G>p.1765fy.PW2317MissensE 38F-IP/PInheritedCS 39c.479+36 A>GInformIAIP/PInheritedCS 40c.594 C>G.6.9951G>p.1765fy.PW231MissensE 38G.16NoneIP/PInheritedCS 39c.594 C>G.6.9951G>p.1765fy.PW231MissensE 38G.16NoneIP/PIp/PIp/PIp/PCS 40c.594 C>G.6.9951G>p.1765fy.PW231Missens <td>CS 26</td> <td>c.205-1G>C</td> <td>-</td> <td>Splicing</td> <td>I 2</td> <td>rs1555605362</td> <td>Р</td> <td>Р</td> <td>Inherited</td>	CS 26	c.205-1G>C	-	Splicing	I 2	rs1555605362	Р	Р	Inherited
CS 29c.7348 C>Tp.R2450*NonsenseE 50rs786202457PPDe novoCS 30c.5305 C>Tp.R1769*NonsenseE 38rs87665714PPDe novoCS 31c.2446 C>Tp.R816*NonsenseE 21rs886041347PPDe novoCS 32c.495_498deITGTp.C167Qfs*10DeletionE 5rs786201874PPDe novoCS 33c.495_498deITGTp.C167Qfs*10DeletionE 5rs786201874PDe novoDe novoCS 34c.495_498deITGTp.C167Qfs*10DeletionE 5rs786201874PDe novoDe novoCS 35c.495_498deITGTp.C167Qfs*10DeletionE 5rs786201874PDe novoDe novoCS 35c.104_105deIGTp.A1071VMisenseE 15rs786201874PDe novoDe novoCS 35c.104_105deIGTp.S35Nfs*2DeletionE 15rs137854557PDe novoDe novoCS 35c.1466A>Gp.Y489CMissenseE 13rs137854557p.PDe novoCS 36c.5294 C>G/c.6951GAp.T17655/p.W2317MissenseE 38 E 4IP/PInheritedCS 36c.5294 C>G/c.6951GAp.T17655/p.W2317MissenseE 38 E 4IP/PInheritedCS 36c.479+36 A>Gp.T17655/p.W2317MissenseE 38 E 4-NoneIP/PInheritedCS 40KoneMone </td <td>CS 27</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>	CS 27								
CS 30c.5305 C>Tp.R1769°NonsenseE 38rs876657714PPInheritedCS 31c.2446 C>Tp.R816°NonsenseE 21rs86041347PPDe novoCS 32c.495_498delTGTTp.C167Qfs*10DeletionE 5rs786201874PPDe novoCS 33c.495_498delTGTTp.C167Qfs*10DeletionE 5rs786201874PPDe novoCS 33c.495_498delTGTTp.C167Qfs*10DeletionE 5rs786201874PDe novoCS 34c.3212 C>Tp.A1071VMissenseE 25-LPLPInheritedCS 35c.104_105delGTp.S35Nfs*2DeletionE 2PDe novoCS 36c.1466A>Gp.Y489CMissenseE 13rs137854557pPDe novoCS 37c.5294 C>G/c.6951 G>Ap.T1765S/p.W2317*MissenseE 38 E 47-LP/PInheritedCS 39c.479+36 A>Gp.T1765S/p.W2317*MissenseE 38 E 47-LP/PInheritedCS 40NoneNoneNoneNoneNoneNoneNoneNoneCS 41c.5446 C>G/c.6951 G>Ap.E517K fs*9DeletionI4LP/PInheritedCS 43c.479+36 A>Gp.E517K fs*9DeletionNoneNoneNoneNoneNoneCS 41c.1542delGp.K2273RMissenseE 45rs10760344ConflictinVLS- </td <td>CS 28</td> <td>c.1527+1G>T</td> <td>-</td> <td>Splicing</td> <td>I 13</td> <td>rs1060500331</td> <td>Р</td> <td>Р</td> <td>De novo</td>	CS 28	c.1527+1G>T	-	Splicing	I 13	rs1060500331	Р	Р	De novo
CS 31c.2446 C>Tp.R816*NonsenseE 21r886041347PPDenoveCS 32c.495_498deITGTp.C167Qfs*10DeletonE 5r5786201874PPDenovoCS 33c.495_498deITGTp.C167Qfs*10DeletonE 5r5786201874PPDenovoCS 34c.3212 C>Tp.A1071VMissenseE 25-LPLPDenovoCS 35c.104_105deIGTp.355Nfs*2DeletonE 25PDenovoCS 36c.104_c6A>Gp.Y489CMissenseE 13r317854557p.0PDenovoCS 37c.5294 C>G.c.6951 GAp.T17655/p.W2317MissenseE 38 E 4-LP/PInheritedCS 38c.5294 C>G.f.c.6951 GAp.T17655/p.W2317MissenseE 38 E 4-LP/PInheritedCS 38c.5294 C>G.f.c.6951 GAp.T17655/p.W2317MissenseE 38 E 4-LP/PInheritedCS 39c.5294 C>G.f.c.6951 GAp.T17655/p.W2317MissenseE 38 E 4LP/PInheritedCS 49c.5294 C>G.f.c.6951 GAp.T17655/p.W2317MissenseE 38 E 4LP/PInheritedCS 49c.5294 C>G.f.c.6951 GAp.T17655/p.W2317MissenseE 38 E 4LP/PInheritedCS 49c.4794 Sp.K144ENoneNoneNoneNoneNoneNoneNoneNoneCS 40c.5818 A>Gp.K144EMisse	CS 29	c.7348 C>T	p.R2450*	Nonsense	E 50	rs786202457	Р	Р	De novo
CS 32c.495_498delTGTp.C167Qfs*10DeletonE fs786201874PPDeletonDeletonCS 33c.495_498delTGTp.C167Qfs*10DeletonE fs786201874PPDelotonDelotonCS 34c.3212 C>Tp.A1071VMissenseE 25-LPLPDelotonDelotonCS 35c.104_105delGTp.S35Nfs*2DeletonE 2PDelotonDelotonCS 35c.1466A>Gp.Y489CMissenseE 13s137854557p.0PDelotonDelotonCS 36c.5294 C>G.c.6951 G>p.1765S/p.W2317MissenseE 38 E 4-LP/PMiseriteCS 37c.5294 C>G.c.6951 G>p.1765S/p.W2317MissenseE 38 E 4-LP/PMiseriteCS 37c.5294 C>G.c.6951 G>p.1765S/p.W2317MissenseE 38 E 4-LP/PMiseriteCS 38c.5294 C>G.c.6951 G>p.1765S/p.W2317MissenseE 38 E 4-LP/PMiseriteCS 49c.5294 C>G.c.6951 G>p.1765S/p.W2317MissenseE 38 E 4-LP/PMiseriteCS 49c.5294 C>G.c.6951 G>p.1765S/p.W2317MissenseF 38 E 4LP/PMiseriteCS 49c.5294 C>G.c.6951 GSp.517K fs* 9DeletionF 14LDeletionDeletionCS 40c.6381 A>Gp.K124MMissenseE 45S10605034MiseriteJD/EDeletionJD/E <td>CS 30</td> <td>c.5305 C>T</td> <td>p.R1769*</td> <td>Nonsense</td> <td>E 38</td> <td>rs876657714</td> <td>Р</td> <td>Р</td> <td>Inherited</td>	CS 30	c.5305 C>T	p.R1769*	Nonsense	E 38	rs876657714	Р	Р	Inherited
CS 33c.495_498deITGTTp.C167Qfs*10DeletionE 5rs786201874PPDeletionDeletionCS 34c.3212 C>Tp.A1071VMissenseE 25-LPLPIheritedCS 35c.104_105deIGTp.S35Nfs*2DeletionE 2PDe novoCS 36c.1466A>Gp.Y489CMissenseE 13rs137854557p.0PDe novoCS 37c.5294 C>G/c.6951 GAp.T1765S/p.W2317MissenseE 38 E 47-LP/PIheritedCS 38c.5294 C>G/c.6951 GAp.T1765S/p.W2317MissenseE 38 E 47-LP/PIheritedCS 39c.479+36 A>Gp.T1765S/p.W2317MissenseE 38 E 47-LP/PIheritedCS 40c.479+36 A>Gp.T1765S/p.W2317MissenseE 38 E 47-LP/PIheritedCS 43c.479+36 A>Gp.T1765S/p.W2317MissenseE 38 E 4-LP/PIheritedCS 43c.479+36 A>Gp.T1765S/p.W2317MissenseE 38 E 4-LP/PIheritedCS 43c.479+36 A>Gp.S17K fs*9DeletionI44-LP/PIheritedCS 44c.4330 A>Gp.K1244EMissenseE 452rs10605034ConflictingVIS-CS 45c.4330 A>Gp.K144EMissenseE 32rs13785455PPCS 45c.4562-Tp.S82FMissenseE 32rs19947470ConflictingP-		c.2446 C>T	p.R816*	Nonsense	E 21	rs886041347	Р	Р	De novo
CS 34c.3212 C>Tp.A1071VMissenseE 25-LPLPInheritedCS 35c.104_105deIGTp.S35Nfs*2DeletionE 2PDe novoCS 36c.1466A>Gp.Y489CMissenseE 13rs137854557pPDe novoCS 37c.5294 C>G/c.6951 G>Ap.T1765S/p.W2317MissenseE 38 E 47-LP/PInheritedCS 38c.5294 C>G/c.6951 G>Ap.T1765S/p.W2317MissenseE 38 E 47-LP/PInheritedCS 39c.479+36 A>Gp.T1765S/p.W2317MissenseE 38 E 47-LP/PInheritedCS 40f.479+36 A>Gp.T1765S/p.W2317MissenseE 38 E 47-LP/PInheritedCS 43c.479+36 A>Gp.T1765S/p.W2317MissenseF 34 E 4-LP/PInheritedCS 43f.479+36 A>Gp.T1765S/p.W2317MissenseF 34 E 4-LP/PInheritedCS 43f.479+36 A>Gp.T1765S/p.W2317MissenseNone-LP/PInheritedCS 43f.479+36 A>Gp.S1776 fs*9DeletionF 14LP/PDe novoCS 44f.681A>Gp.K144EMissenseE 45rs10605034MistenseVISCS 45f.4330 A>Gp.K144EMissenseE 32rs13785455PPCS 45f.245C>Tp.S82FMissenseE 32rs13785450PP<		c.495_498delTGTT	p.C167Qfs*10	Deletion		rs786201874	Р	Р	De novo
CS 35c.104_105delGTp.835Nfs*2DeletinE2PDenoveCS 36c.1466A>Gp.449QMisenseE13r.13785457p.PDenoveCS 37c.5294 C>Gr.6951 GAp.1765S/p.W231*MisenseE38 E4-LP/PInheritedCS 38c.5294 C>Gr.6951 GAp.11765S/p.W231*MisenseE38 E4-LP/PInheritedCS 39c.479+36 A>G-NoneMisenseF43-LP/PInheritedCS 40NoneNoneNoneNoneNoneNoneNoneNoneCS 41c.1542delGp.E517K fs*0DeletinE14LPDelotinCS 42c.6818 A>Gp.K2273RMisenseE43r.10605034OnlicitinVIS-CS 43c.4330 A>Gp.K144EMisenseE32r.13785450PPCS 45c.4525-Tp.82FMisenseE32r.13785450PP		_	p.C167Qfs*10			rs786201874			De novo
CS 36c.1466A>Gp.Y489CMissenseE 13rs13785457pPDe novoCS 37c.5294 C>G/c.6951 G>Ap.T1765S/p.W2317*MissenseE 38 E 47-LP/PInheritedCS 38c.5294 C>G/c.6951 G>Ap.T1765S/p.W2317*MissenseE 38 E 47-LP/PInheritedCS 39c.479+36 A>G-IntroineI 4-LP/PInheritedCS 40NoneNoneNoneNoneNoneNoneNoneCS 41c.1542deIGp.E517K fs*9DeletionE 14-LPDelotonCS 42c.6818 A>Gp.K2273RMissenseE 45rs106050344ConflictingVUS-CS 44c.4330 A>Gp.K144EMissenseE 32rs137854550PPCS 45c.245C>Tp.S82FMissenseE 3rs19947479ConflictingP-			p.A1071V	Missense		-	LP		Inherited
CS 37c.5294 C>G/c.6951 G>Ap.T1765S/p.W2317*MissenseE 38 E 47LP/PInheritedCS 38c.5294 C>G/c.6951 G>Ap.T1765S/p.W2317*MissenseE 38 E 47-LP/PInheritedCS 49c.479+36 A>G-IntronicI 4LP/PInheritedCS 40NoneNoneNoneNoneNoneNoneNoneNoneCS 41c.1542deIGp.E517K fs*9DeletionE 14-LPDenovoCS 42c.6818 A>Gp.K2273RMissenseE 45rs10605034ConflictingVDS-CS 44c.4330 A>Gp.K144EMissenseE 32rs137854550PPCS 45c.245C>Tp.S82FMissenseE 3rs10974729ConflictingP-		-	p.S35Nfs*2	Deletion		-	-		De novo
CS 38c.5294 C>G/c.6951 G>p.T1765S/p.W2317MissensE 38 E 47-LP/PInheritedCS 49c.479+36 A>G-IntoneI4-LPInheritedCS 40NoneNoneNoneNoneNoneNoneNoneNoneCS 41c.1542deIGp.E517K fs*9DeletonE 14-LPDenovoCS 42c.6818 A>Gp.K2273RMissensE 45rs10605034ConflictingVDS-CS 44c.4330 A>Gp.K144EMissensE 32rs13785455PPCS 45c.4525-Tp.S82FMissensE 3rs1994747ConflictingP			•				р		
CS 39c.479+36 A>G–IntronicI 4–LPInheritedCS 40NoneNoneNoneNoneNoneNoneNoneNoneNoneCS 41c.1542deIGp.E517K fs* 9DeletionE 14–LPDenovoCS 42c.6818 A>Gp.K2273RMissenseE 45rs10605034ConflictingVDS–CS 44c.4330 A>Gp.K144EMissenseE 32rs137854550PP––CS 45c.245C>Tp.S82FMissenseE 3rs19947479ConflictingP–						-			
CS 40NoneNoneNoneNoneNoneNoneNoneNoneNoneCS 41c.1542delGp.E517K fs* 9DeletionE 14-LPDe novoCS 42c.6818 A>Gp.K2273RMissenseE 45rs10605034ConflictingVUS-CS 44c.4330 A>Gp.K1444EMissenseE 32rs137854550PP-CS 45c.245C>Tp.S82FMissenseE 3rs19947472ConflictingP-			p.T1765S/p.W2317*	Missense		-			
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.K144E Missense E 32 rs137854550 P P - CS 45 c.245C>T p.S82F Missense E 3 rs199474729 Conflicting P -			-	Intronic		-			
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 -				None		None	None		None
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 -						-			De novo
CS 45 c.245C>T p.S82F Missense E 3 rs199474729 Conflicting P -			p.K2273R				Conflicting		-
			-						-
CS 46 c.4835+1G>A – Splicing I 36 rs1085307819 P P –			p.S82F				-		-
	CS 46	c.4835+1G>A	-	Splicing	I 36	rs1085307819	Р	Р	-

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	
CS 47	c.978delA	p.K326Nfs*50	Deletion	E 9	rs1085307819	Р	Р	-
CS 48	c.2990 G>A	p.R997K	Missense	E 22	rs1555614462	Р	Р	Inherited
CS 49	c.2990 G>A	p.R997K	Missense	E 22	rs1555614462	Р	Р	Inherited
CS 50	c.2446 C>T	p.R816*	Nonsense	E 21	rs886041347	Р	Р	-
CS 51	c.2970_2972 del AAT	p.M992del	Deletion	E 22	rs267606606	Р	Р	-
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	Р	Р	-
CS 56	c.888+1 G>A	-	Splicing	I 8	rs1135402799	Р	Р	-
CS 57	c.4871_4872insAA	p.Y1625Nfs*6	Insertion	E 37	-		Р	-
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	-		Р	Inherited
CS 63	c.2230 G>A	p.W777*	Nonsense	E 20	rs1555613983	Р	Р	De novo
CS 64	None	None	None	None	None	None	None	None
CS 65	c.625 C>T	p.Q209*	Nonsense	E 6	rs786203448	Р	Р	-
CS 66	c.1885 G>A	p.G629R	Missense	E 17	rs199474738	Р	Р	-
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	Р	Р	-
CS 71	c.1397 del T	p.T467Hfs*6	Deletion	E 13	-		Р	-
CS 72	c.3055 G>A	p.V1019I	Missense	E 23	rs1567849826	VUS	Р	-
CS73	c.1754_1757delTAAC	p.T586Lfs*18	Deletion	E16	rs786202782	Р	Р	-
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

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

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