# Neurofibromatosis Type 1 Molecular Diagnosis in Turkish Patients

## Türk Nörofibromatozis Tip1 Hastalarında Moleküler Tanı Sonuçları

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**ABSTRACT**

Neurofibromatosis type 1 (NF1) is a rare autosomal dominant, hereditary tumor-predisposition disorder. NF1 is characterized by multiple cafe-au-lait spots, multiple cutaneous neurofibromas, freckling in the axilla and inguinal area and iris Lish nodules. The prevalence is approximately 1 in 3000 individuals worldwide. In the present study, we aimed to detect the NF1 gene alterations in Turkish NF1 patients using next-generation sequencing (NGS). We analyzed 47 patients for mutations and all of them were unrelated. 27 NF1 mutations were identified. In total, 25 of the 27 mutations were likely pathogenic or pathogenic according to the ACMG criteria. Five of the pathogenic or likely pathogenic variants were novel. This is one of the large NF1 genetic studies in Turkey. We did not determine a genotype-phenotype correlation in this study because of the highly variable expressivity of the NF1 gene. According to our findings each population may have several exon regions that contain recurrent mutations. We suggest that genetic analysis with next-generation tools are more useful and helpful to provide early diagnosis and genetic counseling.

**Key Words:** Neurofibromatosis type 1, Genetic diagnosis, Next generation sequencing

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**ÖZET**

Nörofibromatozis tip 1 (NF1) otozomal dominant kalıtım gösteren nadir bir kansere yatkınlık oluşturan kalıtsal hastalıktır. NF1, ciltte sütlü kahve lekeleri ve fibromatöz tümörler, kolaltı ve inguinal bölgede çillenme ve iriste Lisch nodülü ile karakterizedir. Prevelansı yaklaşık olarak 1\3000 olarak bildirilmektedir. Çalışmamızda, yeni nesil dizileme yöntemi ile Türk Nörofibromatozis hastalarında, NF1 genindeki genomik değişimlerin tespit edilmesi amaçlanmıştır. Birbiri ile akrabalık ilişkisi olmayan 47 hasta çalışılmış olup, 27 NF1 mutasyonu tanımlanmıştır. Toplamda 27 mutasyonun 25'i ACMG kriterlerine göre patojenik yada muhtemel patojenik olarak sınıflandırılmıştır. Bunlardan 5 tanesi ise yeni tanımlanmış mutasyondur. Bu çalışma Türkiye'deki en büyük genetik NF1 çalışmalarından birisidir. NF1 genindeki değişken ekpressiviteden dolayı genotip-fenotip korelasyonunu tanımlamadık. Elde ettiğimiz bulgulara göre her toplum için farklı tekrarlayan mutasyon bölgeleri bulunmaktadır. Son olarak, yeni nesil dizileme araçları, erken genetik tanı ve genetik danışmanlık için son derece yararlı olmaktadır.

**Anahtar Sözcükler:** Nörofibromatozis tip 1, Genetik tanı, Yeni nesil dizileme

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## INTRODUCTION

Neurofibromatosis is a heterogeneous group of hereditary tumor-predisposition disorder that mainly impacts the nervous system and skin. The most common form is neurofibromatosis type 1 (NF1, 96%) that also known as von Recklinghausen disease. The second form is neurofibromatosis type 2 (NF2, 3%) and a lesser-seen form, schwannomatosis. NF1 is one of the most common autosomal dominant human disorder caused by heterozygous mutations of the NF1 gene. NF1 is characterized by multiple cafe-au-lait spots, multiple cutaneous neurofibromas, freckling in the axilla and inguinal area and iris Lisch nodules (1). NF1 is related to many clinical complications that comprise the central nervous system, vascular disease, gastrointestinal, endocrine and skeletal abnormalities. Also, NF1 patients frequently have learning disabilities (2).

The prevalence is approximately 1 in 3000 individuals worldwide irrespective of ethnicity or gender (3). Clinically phenotypic variation is not only among unrelated individuals and among affected within a single-family person even in patients who share the same germline mutation (4). Because, NF1 is a complicated disorder that affects multiple cell types, so many multisystemic complications are seen (5). The diagnosis of NF1 is defined by the National Institutes of Health Consensus in 1988 (6). The diagnostic criteria of NF1 are highly specific and sensitive in adults. The diagnosis can be more problematic in children under 8 years without any other affected family members (7). Therefore, the mutational analysis is more important and essential to make an early and definite diagnosis. Mutational analysis of NF1 is challenging because of the large size of the gene, the existence of multiple pseudogenes (there are 15 pseudogenes), the absence of mutational hotspots and the complex and wide mutational spectrum (8).

NF1 located at 17q11.2. The molecular size of NF1 is 350 kb and contains 60 exons. The most common transcript encodes a 2818 amino-acid polypeptide, neurofibromin (9, 10). Neurofibromin is a Ras guanosine triphosphatase (GTPase) activating protein that inhibits the RAS-MAPK signaling pathway (11). NF1 presents one of the highest mutation rates. To date, 3011 different NF1 variants have been reported in the Human Gene Mutation Database (HGMD) (12).

In the present study, we aimed to detect the NF1 gene alterations in Turkish NF1 patients using next-generation sequencing (NGS) between the 2017-2019 years. We explore NF1 mutation and the obtained data should provide an effective strategy for early and definite diagnosis and genetic counseling.

## MATERIAL and METHODS

### *Patients*

The present study is a retrospective analysis of the neurofibromatosis type 1 patients cohort were from Ankara Dışkapı Yıldırım Beyazıt Training and Research Hospital, Medical Genetics Clinic, between 2017 and 2019. The patients were referred to our department as they were suspected of NF1. We analyzed 47 patients for mutations and all of them were unrelated. Twenty-seven NF1 mutations were identified. The ethical committee of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital approved the study (2020-03/582).

### *DNA Sequencing*

Blood samples were collected into EDTA tubes. DNA of patients extracted by QIAcube® automated DNA isolation system (Qiagen Inc. Mississauga, ON, Canada). Isolated DNA samples were stored at −20°C. Before sequencing, the DNA concentration and quality were measured by NanoDrop (ND-1000) spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA) for OD260/OD280, 1.8–2.0.

### *Genetic Testing*

NF1 MASTR™ Dx (Multiplicom, Niel, Belgium) and Ion AmpliSeq NF1 (Life Technologies, Carlsbad, CA, USA) were used for targeting NF1 coding regions. Amplicon products were dual barcoded for sample identification. The sequencing was performed on the Illumina MiSeq system (Illumina Inc., San Diego, CA, USA).

The data analyses were performed Sophia DDM software (Sophia Genetics, Saint-Sulp) for Multiplicom kit.. Sanger validation was performed for: homopolymer regions, low quality variants, insertions and/or deletions, splice site alterations and novel variants.

### *Variant Classification*

The recent ACMG/AMP guideline for standardized variant interpretation in Mendelian disorders was used for classification. Pathogenic variants are well-established disease-causing DNA changes in the in-house database and/or literature. Likely pathogenic variants are considered the probable cause of the disease or the effect on the protein function is predicted to be likely deleterious (>90% probability to cause the disease). VUS alterations are genetic variants with unknown or questionable impact on the disease. These variants are typically very rare and predicted to be deleterious.

## RESULTS

NF1 gene shows one of the highest mutation rates, and different populations present with different exon regions of recurrent mutations (4).

In our study, 27 mutations were found in 47 patients who were clinically suspected to have neurofibromatosis type 1 (57%). The identified mutations were distributed across exon 9 to 49 of the NF1 gene. The list of all genomic variations detected (pathogenic or not) is reported in Table 1. We observed 8 missense variants, 8 nonsense variants, 7 splice site variants, and 4 frameshift variants. The majority were nonsense and missense mutations. In total, 25 of the 27 mutations were likely pathogenic or pathogenic according to the ACMG criteria. Only two genomic variations were defined as VUS. Five of the pathogenic or likely pathogenic variants were novel (Table 1).

## DISCUSSION

The present study was performed to identify the mutational spectrum of the NF1 gene in Turkish patients, which would allow rapid and economical screening of certain selected exons. Definition of NF1 mutation spectrum is significant for the understanding of the molecular mechanisms, for the clinical follow-up and genetic counseling (13). However, only three recurrent mutations were found in 27 Turkish NF1 patients (Patient number 11 was used to identify two sisters patients, so there were totally 28 patients). This finding demonstrates the extensive distribution of mutations and the absence of a mutational hotspot in the NF1 gene. Some previous studies put forward that particular regions may be mutation hotspots (14, 15). The results were conflicting among the studies, even though there were some overlapping exons including exon 1, 16, 27a, 29, 37, as well as intron 13 (4).

In our patient cohort, c.1541-1542delAG found in two patients (patient number of 4 and 5) and c.1646 T>C was also observed in two patients (patient number of 6 and 7). Another recurrent mutation was c.2409+1 G>C which found in two sisters (patient number 10) and another unrelated patient (patient number 11). The most frequent mutation region was exon 30 (In three patients). According to our findings, exon 30 of the NF1 gene may be a mutation-prone region in the Turkish population in spite of the need for further research. These results suggest that each population may have several exon regions that contain recurrent mutations.

Moreover, we detected some recurrent mutations in both our study and the previous two Turkish study Ulusal et al. (16) and Terzi et al (10). First, c.1541-1542delAG and c.3709-2A>G were reported by Ulusal et al. like our study does. Other recurrent mutation was c.6709C>T which was defined by Ulusal et al. Terzi et al. and us. These data demonstrate that these mutations may be the NF1 gene hotspots region in Turkish patients.

Our study is one of the largest studies of NF1 mutations in Turkey. We detected 27 mutations of the whole NF1 coding region and exon-intron boundaries. We did not determine a genotype-phenotype correlation in this study because of the highly variable expressivity of the NF1 gene. Finally, we suggest that genetic analysis with next-generation tools are more useful and helpful to provide early diagnosis and genetic counseling. So it can be used as the first-choice method for an effective strategy.

**Table 1:** The list of all genomic variations detected (pathogenic or not) in our patients

| Patient number | Gender | Age | Exon number | Nucleotide change | Affected protein | Mutation type | Pathogenicity (ACMG criteria) | Novelty |
|---|---|---|---|---|---|---|---|---|
| 1 | M | 31 | 9 | c.935delG | p.Gly312Glufs | Frameshift | Likely Pathogenic | Novel |
| 2 | F | 15 | 11 | c.1213A>G | p.Thr405Ala | Missense | VUS | |
| | | | 24 | c.3118A>G | p.Lys1040Glu | | | |
| 3 | M | 4 | 11. intron | c.1260+1delG | - | Splice site | Likely pathogenic | Novel |
| 4 | M | 7 | 14 | c.1541_1542delAG | p.Gln514Argfs*43 | Frameshift | Pathogenic | |
| 5 | F | 26 | 14 | c.1541_1542delAG | p.Gln514Argfs*43 | Frameshift | Pathogenic | |
| 6 | M | 3 | 15 | c.1646T>C | p.Leu549Pro | Missense | Likely Pathogenic | |
| 7 | F | 11 | 15 | c.1646T>C | p.Leu549Pro | Missense | Likely Pathogenic | |
| 8 | M | 17 | 18 | c.2041C>T | p.Arg681TEer | Nonsense | Pathogenic | |
| 9 | M | 1 | 19 | c.2288T>G | p.Leu763Arg | Missense | Likely Pathogenic | |
| 10 | M | 2 | 20. intron | c.2409+1 G>C | - | Splice site | Pathogenic | |
| 11 | F-F | 2-6 | 20. intron | c.2409+1G>C | - | Splice site | Pathogenic | |
| 12 | M | 9 | 27. intron | c.3709-2A>G | - | Splice site | Likely Pathogenic | |
| 13 | M | 12 | 30 | c.3986C>A | p.Ser1329Ter | Nonsense | Pathogenic | |
| 14 | F | 17 | 30 | c.4021C>T | p.Gln1341Ter | Nonsense | Pathogenic | |
| 15 | F | 47 | 30 | c.4084C>T | p.Arg1362Ter | Nonsense | Pathogenic | |
| 16 | F | 4 | 31 | c.4267A>G | p.Lys1423Glu | Missense | Pathogenic | |
| 17 | F | 12 | 32. intron | c.4270-1G>C | - | Splice site | Pathogenic | Novel |
| 18 | F | 8 | 32. intron | c.4270-2A>G | - | Splice site | Pathogenic | |
| 19 | M | 17 | 32 | c.4277A>G | p.Gln1426Arg | Missense | Likely Pathogenic | |
| 20 | F | 23 | 36 | c.5035_5038delinsTTC | p.Leu1679Phefs*10 | Frameshift | Pathogenic | Novel |
| 21 | F | 13 | 37 | c.5242C>T | p.Arg1748Ter | Nonsense | Pathogenic | |
| 22 | F | 10 | 38 | c.5489G>T | p.Arg1830Leu | Missense | Likely pathogenic | |
| 23 | M | 9 | 39 | c.5839C>T | p.Arg1947Ter | Nonsense | Pathogenic | |
| 24 | F | 17 | 40 | c.6085-2A>G | - | Splice site | Pathogenic | Novel |
| 25 | M | 4 | 42 | c.6410C>G | p.Ser2137Ter | Nonsense | Pathogenic | |
| 26 | F | 6 | 44 | c.6709C>T | p.Arg2237Ter | Nonsense | Likely Pathogenic | |
| 27 | F | 0 | 49 | c.7247C>A | p.Ala2416Asp | Missense | VUS | |

**Conflict of interest**

No conflict of interest was declared by the authors.

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