Original Article

Neurofibromatosis Type 1 and Type2 (NF1 and NF2): Molecular Genetic Profiles of the Patients.

Nörofibromatozis Tip 1 ve Tip2 (NF1 ve NF2): Hastaların Moleküler Genetik Profilleri

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ABSTRACT

Introduction: Neurofibromatosis type I and type 2 (NF1 and NF2) are two rare autosomal dominant neurocutaneous genetic disorders that are diagnosed based on clinical diagnostic criteria of the National Institutes of Health Consensus. Due to increased malignancy risks and age-related penetrance, NGS-based diagnosis methods should be used for early diagnosis. However, molecular diagnosis of the NF1 and NF2 genes are challenging owing to the large size of genes, and the lack of mutation hotspots. In this study, we present 67 patients between 2016-2019 who were investigated for NF1 and NF2 genes with the next generation sequencing (NGS). We aimed to show the effectiveness of NGS-based molecular genetic diagnoses and contribute to establishing the variant spectrum in the Turkish population.

Materials and methods: Patients who met the diagnostic criteria were included in this study. After DNA extraction from peripheric blood samples, an NGS-based panel that includes both NF1 and NF2 genes was performed. Results were evaluated using ACMG 2015 criteria and in silico bioinformatics tools.

Results: Thirtynine of 67 total patients revealed various variants (39/67, ~58%). The variant distribution was as follows; 15 frameshift, 8 nonsense, 7 missense, 6 splice sites, and three insertion/deletion. Novel variants ratio was 23/39 (22 NF1, 1 NF2, ~59%). The classification according to clinical significance was as follows: 23 pathogenic, 14 likely pathogenic, and one VUS according to ACMG 2015 criteria.

Discussion: Our results suggested that a genetic screening using an NGS panel is more useful and helpful to provide early diagnosis and genetic counseling and have a positive impact on patient follow-up.

Keywords: Neurofibromatosis type 1, Neurofibromatosis type 2, Next-generation sequencing

ÖZET

Giriş: Nörofibromatozis tip I ve tip 2 (NF1 ve NF2), Ulusal Sağlık Konsensüsü Enstitüleri'nin klinik tanı kriterlerine göre teşhis edilen iki nadir otozomal dominant nörokutanöz genetik hastalıktır. Artan malignite riskleri ve yaşa bağlı penetrans nedeniyle erken tanı için NGS temelli tanı yöntemleri kullanılmalıdır. Bununla birlikte, NF1 ve NF2 genlerinin moleküler teşhisi, genlerin büyük boyutu ve mutasyon noktalarının olmaması nedeniyle zordur. Bu çalışmada 2016-2019 yılları arasında yeni nesil dizileme (NGS) ile NF1 ve NF2 genleri araştırılan 67 hasta sunuldu. NGS temelli moleküler genetik tanıların etkinliğini göstermeyi ve Türk popülasyonunda varyant spektrumunun oluşturulmasına katkıda bulunmayı amaçladık.

Gereç ve yöntemler: Tanı kriterlerini karşılayan hastalar dahil edildi.
Periferik kan örneklerinden DNA ekstraksiyonundan sonra, hem NF1 hem de NF2 genlerini içeren NGS tabanlı bir panel gerçekleştirildi. Sonuçlar ACMG 2015 kriterleri ve in silico biyoinformatik araçları kullanılarak değerlendirildi.

Bulgular: Toplam 67 hastanın 39'unda çeşitli varyantlar saptandı (39/67, ~%58). Varyant dağılımı şu şekildeydi; 15 çerçeve kayması, 8 anlamsız, 7 yanlış anlamlı, 6 splays bölge ve üç insersiyon/delesyon.

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Yeni varyant oranı 23/39 (22 NF1, 1 NF2, ~%59) idi. Klinik öneme göre sınıflandırma şu şekildeydi: ACMG 2015 kriterlerine göre 23 patojenik, 14 olası patojenik ve bir VUS.
Tartışma: Sonuçlarımız, bir NGS paneli kullanılarak yapılan genetik taramanın, erken tanı ve genetik danışmanlık sağlamak için daha yararlı ve yararlı olduğunu ve hasta takibini olumlu yönde etkilediğini göstermiştir.

Anahtar kelimeler: Nörofibromatoz tip 1, Nörofibromatoz tip 2, Yeni nesil dizileme

Introduction

Neurofibromatosis type I and type 2 (NF1 and NF2) are autosomal dominant neurocutaneous genetic disorders that affected individuals leading to increased susceptibility to the development of benign and malignant tumors. The estimated worldwide incidence of NF1 and NF2 are 1 in 2,500 to 1 in 3,000 individuals [1, 2] and 1 in 25,000 live births respectively [3]. While NF1 is characterized by cafe-au-lait spots, Lisch nodules in the eye, and fibromatous tumors of the skin, NF2, is characterized by tumors of the eighth cranial nerve (usually bilateral), meningiomas of the brain, and schwannomas of the dorsal roots of the spinal cord. To establish the diagnoses, clinical diagnostic criteria determined by the NIH are used for both diseases [4, 5].

Although the most common benign tumors in NF1 patients are neurofibromas, they can also be seen in other neoplasms such as optic nerve gliomas and brain tumors [6]. A variety of other tumors, including rhabdomyosarcomas, pheochromocytomas, gastrointestinal stromal tumors, glomus tumors, and retinal vaso-proliferative tumors, are more common in individuals with NF1 compared with the normal population [7].

In NF2, the mean age of onset of symptoms in individuals are variable (birth to 70 years). Almost all affected individuals develop bilateral vestibular schwannomas by age 30. Schwannomas, meningiomas, ependymomas, and (very rarely) astrocytomas are observed in individuals with NF2 as tumors that affect other cranial and peripheral nerves.

NF1 is caused by heterozygote pathogenic variants which leads to loss of function of the tumor suppressor NF1 gene (Neurofibromin 1; MIM no. 613113). NF1 gene which is located at 17q11.2 and contains 58 exons, encodes a 2839 amino acid peptide called neurofibromin. Neurofibromin is a Ras guanosine triphosphatase (GTPase) activating protein that reflects its role as an important negative regulator of the cellular RAS-MAPK (mitogen-activated protein kinases) signaling pathway. Thus, in the case of loss of function, the active GTP-bound RAS form increases cellular levels and results in uncontrolled cell growth and potentially tumorigenesis.

NF2 gene is located on chromosome 22q12.2 and contains 17 exons that encode for a 69 kDa protein product called merlin (moesin-ezrin-radixin-like protein) or schwannomin [8]. Merlin is a tumor suppressor protein located at the cell membrane-cytoskeleton interface which inhibits cell growth [9]. The lacking growth-inhibitory activity of Merlin leads to disrupting tumor suppression. On the NF2 mutation database, 159 unique variants have been reported. (Leiden Open Variation Database, LOVD: www.lovd.nl/NF2)

Compared to other genetic diseases in humans, the incidence of pathogenic variants in the NF1 gene is quite high (10). Also, the de novo pathogenic variant is exhibited in almost 50% of NF1 patients. More than 3700 variants have been reported. Among them, 64% are substitutions, 23% are deletions and eight are duplications. These variants are classified as 44% missense, 37% frameshift, and 12% stop changes according to their effects on protein level (Leiden Open Variation Database, LOVD: www.lovd.nl /NF1).

Because of their increased malignancy risks, molecular genetic diagnosis of NF1 and NF2 is very important in terms of enabling early diagnosis, follow-up, and treatment. However, molecular diagnosis of the NF1 gene is difficult due to the large size of the gene, the distribution of variants across the

entire gene, the presence of many highly identical pseudogenes, and a wide spectrum of variants [11, 12]. The increasing use of next-generation sequencing (NGS) and specially customized targeted gene panels are facilitating accurate and fast molecular genetic diagnosis. Due to the phenotypic overlapping of NF1 and NF2 diseases, an NGS panel that includes both the NF1 and NF2 genes is a time- and cost-saving manner.

In this study, the results of patients who applied to our clinic with the pre-diagnosis of Neurofibromatosis between 2016-2019 and were investigated for NF1 and NF2 genes with the next-generation sequencing (NGS) method were evaluated. We aimed to show the effectiveness of NGS-based molecular genetic diagnoses and the obtained data are intended to help provide an effective strategy for early and definitive diagnosis and genetic counseling of the NF1 and NF2 patients. Our results suggested that a genetic screening using an NGS panel is more useful and helpful to provide early diagnosis and genetic counseling and has a positive impact on patient follow-up.

Materials and Method

All of the procedures were carried out in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from the parents of the participants for molecular genetic analysis and the publication of patient data prior to their enrolment in the study. This study was conducted after approval was given by the Ankara City Hospital Ethics Committee (Document Number: E1-2644/2022).

Patients

Between 2016 and 2019, 67 index cases were referred to our clinic for molecular diagnosis of NF1 and NF2. Clinical diagnoses were executed according to the diagnosis criteria of NIH.

In this study, we analyzed the patients who were referred to our department with an NGS-based panel that includes both NF1 and NF2 gene In this study, we analyzed the patients who were referred to our department with an NGS-based panel that includes both NF1 and NF2 gene

Genetic analyses

Genetic analyses were performed for diagnosis with a parental informed consent form. The DNA was extracted from peripheral blood by QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A targeted NGS in-house gene panel was designed for the genes associated with NF1 and NF2 genes. NGS analyses were performed on the MiSeq system (Illumina, San Diego, CA, USA).

In silico bioinformatics analyses

Potential functional effects of novel missense variants were predicted using the Alamut® Visual 2.4 Software (SIFT, Polyphen-2, and Variant Taster). information about the location of AA and predicted transmembrane domains were taken from Uniprot (http://www.uniprot.org/).

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 impacts on the disease. These variants are typically very rare and predicted to be deleterious.

Results

Between 2016 and 2019, 67 patients were referred to our clinic for genetic diagnosis of Neurofibromatosis. We performed NGS-based molecular genetic analysis on 67 patients which clinically suspicious of the diseases according to NIH criteria. The average age of the patients was ~21. While the result was normal in 28 patients, various variants were detected in 39 patients (39/67, ~%58) (Figure 1). In the positive group, the

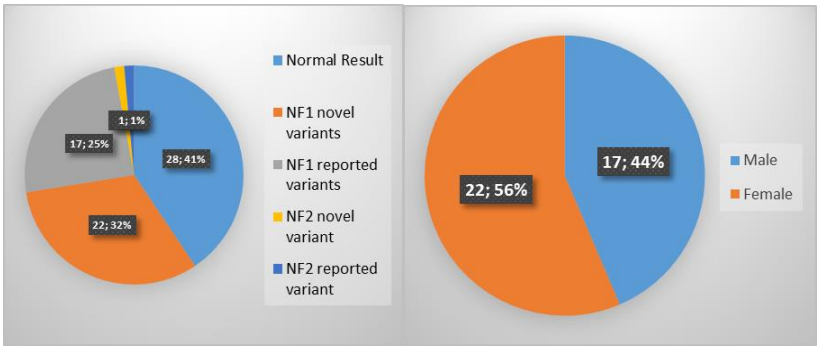

Figure 1. The detection rate of the molecular genetic testing and the Male/female ratio of the patients with the positive genetic result.

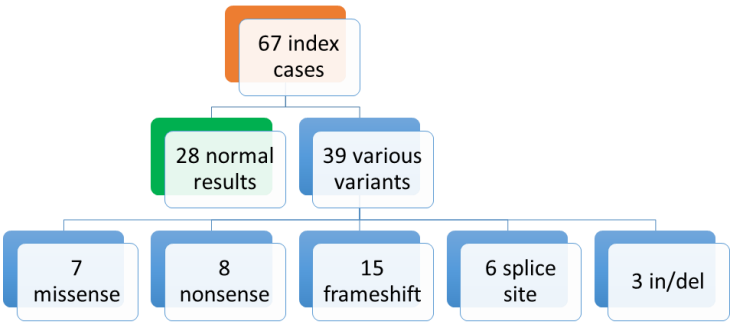

Figure 2. Variant distribution according to the molecular type.

male and female ratio was 17/38(44%) and 22/38 (%56) respectively (Figure 1). The variant distribution was as follows; 15 frameshift, eight nonsense, seven missense, six splice sites, and an insertion/deletion (Figure 2). The number of novel variants was 23 (22 NF1, 1 NF2) which is quite high (23/39, ~%59). The classification according to clinical significance was as follows:23 pathogenic, 14 likely pathogenic, and one VUS according to ACMG 2015 criteria (Table 1).

Discussion

In this study, we performed NGS-based molecular genetic analyses on 67 patients who were referred with clinical suspicion of NF1 and NF2. Results revealed 39 different variants from 39 patients (Table 1). These variants were heterogeneous and distributed within the gene without clustering. A significant difference was not observed between the sexes (Figure 1). In our study, the NF1 or NF2 variant was detected in 58.2% (39/67) of the patients who met the clinical diagnostic criteria. It showed that the detection rate of variants in the NF1 gene is very high in patients which clinically diagnosed with neurofibromatosis according to NIH criteria [4]. Although our variant detection rate is very high, some previous studies combining RNA and MLPA analyzes have reported higher detection rates of up to 92% in patients. [13, 14]. This may be because the additional methods they used also revealed variants and large deletions occurring in deep intron regions. Therefore, RNA studies and MLPA analyses should be recommended in patients with normal NGS analysis.

NF1 and NF2 are two progressive disorders complicated by the variability of disease expression and age-related penetrance. Therefore, molecular genetic analyses becoming more important for these particular diseases. However, molecular diagnosis of both NF1 and NF2 genes is difficult due to the gene's large size and absence of hotspots, and the wide diversity of mutations [15]. In our study, variants also did not show hot-spot

Table 1. Variant distribution and classification among the patients.

Patient	Age(Year)	Gender	Gene	Results	Mutation Type	Variant Id	Classification (Acmg 2015)
1	10	M	*NF1*	c.2T>G (p.Met1Arg) (Start loss)	Missense	rs886041346	P
2	21	F	*NF1*	c.1682G>A (p.Trp561*)	Nonsense	Novel	P
3	6	M	*NF1*	c.4558C>T (p.Gln1520*)	Nonsense	rs1060500242	P
4	21	F	*NF1*	c.574C>T (p.Arg192*)	Nonsense	rs397514641	P
5	26	M	*NF1*	c.3496+1G>A	Splice site	CS072245	LP
6	55	F	*NF1*	c.2033delC (p.P678fs*10)	Frameshift	rs747195556	P
7	45	F	*NF1*	c.7152_7153insT (p.Asn2385*)	Nonsense	Novel	P
8	19	M	*NF1*	c.3596_3599delCAGT (p.Thr1199Asnfs*15)	Frameshift	Novel	LP
9	5	F	*NF1*	c.1842_1845+1delTAAGG (p.Ala548_Lys615de)	Frameshift	rs1135402822	P
10	53	M	*NF1*	c.3376C>T p.Gln1126*	Nonsense	Novel	P
11	11	M	*NF1*	c.5766_5767delinsTC (p.Thr1902Pro)	Missense	Novel	LP
12	35	F	*NF1*	c.1754_1757delTAAC (p.T586Vfs18)	Frameshift	rs1277850570	P
13	9	F	*NF1*	c.6687G>A (p.Trp2229*)	Nonsense	Novel	P
14	24	M	*NF1*	c.1033_1062+6del36 (p.Leu345_Lys354del)	Frameshift	Novel	P
15	26	M	*NF1*	c.5609G>A	Missense	rs786202112	P
16	38	F	*NF1*	c.3457_3460delCTCA (p.Leu1153Metfs*4)	Frameshift	rs1321848637	P
17	18	F	*NF1*	c.6319_6321delGTT (p.Val2106del)	Small deletion	Novel	LP
18	21	F	*NF1*	c.1246C>T (p.Arg416*)	Nonsense	rs764079291	P
19	8	F	*NF1*	c.4600C>T	Missense	rs760703505	P
20	14	F	*NF1*	c.3430_3431insCACT (p.Cys1144Serfs*52)	Frameshift	Novel	LP
21	46	F	*NF1*	c.4578-14T>G	Splice site	Novel	VUS
22	18	F	*NF1*	c.4687_4688insTA (p.Asn1563Ilefs*12)	Frameshift	Novel	LP
23	21	F	*NF1*	c.7438_7447delCATGGTGACC (p.His2480Leufs*6)	Frameshift	Novel	LP
24	10	F	*NF1*	c.3827G>A (p.Arg1276Gln)	Missense	rs137854556	P
25	14	F	*NF1*	c.3430_3431insCACT (p.Cys1144Serfs*52)	Frameshift	Novel	LP
26	11	M	*NF1*	c.1246C>T	Missense	rs764079291	P
27	15	M	*NF1*	c.1527+4_1527+7del	Splice site	Novel	LP
28	17	F	*NF1*	c.3656_3658del (p.Gly1219del)	Small deletion	rs1555615077	VUS
29	17	F	*NF1*	c.3922del, (p.Val1308*)	Nonsense	Novel	LP
30	18	M	*NF1*	c.1845+2T>G	Splice site	Novel	LP
31	13	M	*NF1*	c.5425C>T (p.Arg1809Cys)	Missense	rs797045139	P
32	10	M	*NF1*	c.4595del, (p.Pro1532Leufs*21)	Frameshift	Novel	LP
33	19	M	*NF1*	c.7323_7324insA (p.Leu2442Thrfs*3)	Frameshift	Novel	P
34	17	M	*NF1*	c.1453del, (p.Glu485Argfs*13)	Frameshift	Novel	P
35	7	F	*NF1*	c.2156del, (p.Ile719Thrfs*29)	Frameshift	Novel	P
36	13	M	*NF1*	c.6833_6841delCCAAATTACinsTTT (p.Thr2278_Gln2281delinsIle*)	Deletion/intertion	Novel	LP
37	6	M	*NF1*	c.499_502del, (p.Cys167Glnfs*10)	Frameshift	rs786201874	P
38	20	F	*NF2*	c.676-1G>A	Splice site	CS982290	LP
39	17	F	*NF2*	c.241-2A>G	Splice site	Novel	P

M: male, F: female, *NF1*(NM_001042492): neurofibromin 1, *NF2*(NM_000268): neurofibromin 2, P: pathogenic, LP: likely pathogenic, VUS: variant of unknown significance

properties. The novel variant rate was quite high in the patients (22/39, 56.4%) comparing previous similar studies [13]. However, molecular genetic testing is important for early diagnosis because of the clinical variability and age-related penetrance. [16]. In recent years, NGS has provided rapid and cost-effective molecular diagnosis options, especially in the molecular diagnosis of genetically heterogeneous and clinically overlapping inherited diseases such as NF1 and NF2 [17].

Our study is one of the largest studies from Turkey, The variants distributed across the entire gene did not reveal any hot spot region. Another study from Turkey reports similar findings [18]. This may indicate the conclusion that the disease does not show a hotspot zone in the Turkish population as it does in other populations. According to the NF1 database [19], our non-sense and frameshift mutation rates are also high. This may be because the patients we studied were from different ethnic groups and there were not many studies in Turkey. Relatively fewer mutations were observed in the NF2 gene, as expected. Further studies are needed for more information.

Finally, our study was to show molecular genetic analysis allows early diagnosis while

the clinic is not yet fully established. In this way, early diagnosis and genetic counseling are available. Considering the increased risk of malignancy in both diseases, early diagnosis becomes very important for screening the patient who is at malignancy risk. We suggest that genetic analysis with next-generation tools be used as the first-choice method for an effective strategy. This study also contributes to the establishment of the NF1 and NF2 variant spectrum in the Turkish population, which has been under-studied.

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