ORIGINAL ARTICLE

Evaluation of ocular and genetic findings in patients with Neurofibromatosis Type 1

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Abstract

Neurofibromatosis type 1 (NF1) is an autosomal dominantly inherited disease affecting multiple organ systems and showing many different clinical symptoms. The severity of the disease varies from person to person and progresses gradually over the years. In this study, 17 NF1 patients who had a definite diagnosis were evaluated in terms of genetic, ophthalmological, and nervous system investigations. Approximately 5000 patients who visited medical genetics clinic between 2012 and 2022 are recorded in our archive. In 17 of these patients, a definitive genetic diagnosis was made. In the course of the study, the researchers collected some clinical parameters such as antenatal, intrapartum, and postpartum history and family history. In the family history, the researchers did a detailed pedigree with at least 3 generations of analysis, questioned parental kinship, looked for similar members in families, and identified inheritance patterns of the disorder. Peripheral venous blood samples were taken from the patients and sent to a commercial laboratory for gene panels or WES while the karyotyping was carried out in our laboratory. After obtaining the definitive genetic diagnosis of all patients, we compiled a table with the other parameters we questioned. This study presented the genotype and phenotype findings of NF1 patients. Ophthalmological symptoms in patients were also examined. These new-generation genetic disease diagnosis methods can be routinely used in clinical practice by medical geneticists. The diagnosis of a disease is one step ahead of its treatment. Because if the necessary diagnosis is not made, treatment of the disease is not possible. While this situation was more difficult in the past, nowadays, with the developing technology, diseases can be diagnosed more easily. In NF1 disease, more information can be obtained as a result of genetics, imaging, and examinations of other branches.

Keywords: Genetic analyses, neurofibromatosis type 1, ophthalmology

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Introduction

Neurofibromatosis type 1 (NF1) is an autosomal dominant disease that affects multiple organ systems and has many variable clinical presentations. NF1 is characterised by neurofibromas. These are nerve sheath tumors that form in close association with spinal, peripheral, or cranial nerves. Other features include pigment changes, low-grade glioma, skeletal dysplasia, and involvement of many other organ systems. Although the specific manifestations, rate of progression, and severity of complications vary widely, the disease is progressive over a lifetime. Currently, there is no definitive treatment. Clinical management is typically limited to surveillance and symptomatic treatment, usually surgical, for specific complications [1].

The gene that causes NF1 was identified in 1990, and its function and role in tumourigenesis and other features of NF1 are being studied intensively. As our understanding of the mechanisms underlying the pathogenesis of the clinical features of NF1 has improved, a number of targeted therapies have emerged. These are now being evaluated in preclinical models and phase II clinical trials. For people with NF1, the emergence of new treatments aimed at improving their quality of life (QOL) is a time of great hope [2]. The aim of this study is to evaluate these patients with a definitive genetic diagnosis and to publish the results in the literature.

Neurofibromatosis Type 1 and Genetics Diagnosis

NF1 is caused by changes in the NF1 gene, which is located on chromosome *17q11.2*. Many lines of evidence suggest that NF1 is a tumor suppressor gene. Inactivation of both NF1 alleles would reduce control of cell proliferation and lead to tumorigenesis. The function of the NF1 gene product, neurofibromin, is to stimulate RAS protein GTPase activity and act as a negative regulator of the cellular Ras/MAPK (mitogen-activated protein kinases) pathway [3]. So far, more than 1.000 pathogenic allelic variants have been identified in the NF1 gene. Most NF1 mutations are single-base substitutions, insertions, or deletions. Other mutations are single- or multi-exon deletions or duplications and microdeletions encompassing NF1 and its neighboring genes [4].

The development of next-generation sequencing (NGS) technologies, which allow rapid identification of mutations and high-risk aleads that cause the disease, has recently entered NF1 diagnostics. Clinical diagnosis and molecular relations should be better understood. Therefore, a comprehensive genetic characterization of this disorder in a clinical environment will help to better understand the process [5].

Neurofibromatosis Type 1 and Oftalmological Clinical Features

It is the most common phakomatosis with autosomal dominant genetic inheritance. Sharply circumscribed, dome-shaped pigmented Lisch nodules, which are melanocytic hamartomas in the iris stroma, are the most common ocular involvement in NF1 [6]. Iris nevi, which can be confused with Lisch nodules, are observed as flat or slightly elliptical, densely pigmented lesions with blurred borders [7]. Lisch nodules are more common in the lower half of the iris than in the upper half due to increased exposure to sunlight and increased pigmentation [8]. In a study examining the distribution of Lisch nodules according to age, it was found that Lisch nodules were found in 5% under 3 years of age, 42% in 3-4 years of age, 55% in 5-6 years of age and all individuals over 21 years of age [7]. Therefore, the absence of Lisch nodules in children, which are included in the diagnostic criteria for NF1, does not rule out the diagnosis of NF1.

Lisch nodules, which are frequently seen bilaterally and in large numbers, are observed in one eye in segmental neurofibromatosis Cases in the half of the face where cutaneous lesions are present [9]. Lisch nodules do not require treatment because they are benign and do not cause visual loss [10]. Optic pathway gliomas are the most important lesions that cause visual loss. They are included in the NF1 diagnostic criteria and are seen in 5% to 25% of patients [11,12]. Gliomas, which are typically low-grade pilocytic astrocytomas involving the optic nerve, usually have an asymptomatic course, but depending on the site of involvement, patients may experience

decreased visual acuity, color vision impairment, visual field defects, relative afferent pupillary defects, proptosis, strabismus, nystagmus, and puberty precocity [10,13]. Since the risk of optic glioma decreases in children older than 8 years of age, it is recommended that detailed eye screening be performed every year until the age of 8 years and every two years between the ages of 8-18 years [14,15].

In patients with NF1-associated optic gliomas, it is important to follow up every three months in the first year after diagnosis, with an interval in the following period [14]. Diagnostic brain or orbital magnetic resonance imaging (MRI) is recommended to be performed by the ophthalmologist when a suspicious lesion is detected and simultaneously or less frequently with ophthalmological controls during follow-up [14]. Chemotherapeutic drugs such as vincristine or cisplatin may be used in symptomatic optic pathway gliomas [16]. Radiotherapy is generally not preferred in very young children because of the risk of neurological complications, vascular pathologies such as Moyamoya disease, and the risk of secondary malignancy [17,18].

Surgical treatment is generally not recommended, although it has been reported that surgical intervention can sometimes be performed to remove severe proptosis or dense chiasmal gliomas[18]. Rapamycin, a specific mTOR inhibitor, is thought to reduce astrocyte growth in vitro and may be an alternative in the treatment of optic gliomas and plexiform neurofibromas [19,20].

Neurofibromas are benign skin tumors that may involve the eyelid, brow, and orbit and are seen in less than 10% of patients with NF1 [6,12]. Neurofibromas that are soft on palpation and have a worm bag sensation are seen as an *S*-shaped deformity of the eyelid leading to asymmetric ptosis [6,11]. Neurofibromas of the nodular type are more common, whereas neurofibromas of the plexiform type are more clinically significant and can lead to proptosis, strabismus, amblyopia, and bone deformity [6,10]. Sphenoid bone dysplasia, which is associated with orbital plexiform neurofibromas, can be observed in NF 1 and as a result, the temporal lobe protrudes into the orbit, resulting in pulsatile proptosis synchronized with the heartbeat [11].

Surgical interventions are challenging due to the tumor location and high bleeding risks of neurofibromas, and therefore agents such as kinase inhibitors, mTOR inhibitors, and fibroblast inhibitors are mainly recommended for treatment [12-21]. Glaucoma, which can develop in patients with NF1 by many pathogenic mechanisms, is not common but may develop at birth or in early childhood [22]. The most common cause is disruption of aqueous drainage after neurofibromatous infiltration and obstruction, while secondary angle closure, synechial angle closure, and neovascular glaucoma may develop with ciliary body infiltration [22,23].

Corneal nerve dysfunction and thickening of the corneal nerves leading to corneal epithelial changes are thought to be due to genetic changes in axons and Schwann cells [24,25]. Although choroidal nodules can be difficult to detect by fundus examination and fluroscein angiography, indocyanine green angiography shows delayed perfusion in the choriocapillaries surrounding the nodule [26].

In NF1 patients with less retinal involvement, multiple retinal capillary haemangiomas, astrocytic hamartomas, and combined retinal-retinal pigment epithelium hamartomas may be seen [27]. In addition, epiretinal membrane, sectoral retinitis pigmentosa, and myelinated nerve fibers may be seen [28].

Material and Methods

This study was conducted with the approval of Afyonkarahisar Health Sciences University Ethics Committee (2022/12). 17 patients diagnosed with NF1, who were definitively diagnosed by our medical genetics clinic, were included in the study. We collected some clinical parameters, including the patient's prenatal, natal, and postnatal history, as well as family history, during our research. The family history included a detailed genealogy of at least three generations, asking about parental relationships, looking for similar members in the family, and identifying patterns of inheritance of the condition. While karyotyping was done in our

lab, the researchers collected peripheral venous blood samples from patients and sent them to a commercial lab for gene panels or WES. After obtaining the definitive genetic diagnosis of all patients, the researchers compiled a table with the other parameters the researchers questioned.

In NF1 disease, the characteristic findings of café au lait spots, Lisch nodules, neurofibromas, optic gliomas, osseous lesions, and family history are re-evaluated in patients. Hospital records were therefore used to obtain patients' presenting symptoms, family history, and MRI findings. Dysmorphic facial features were also identified from the patients' photographs. In this process, "The Elements of Morphology: Human Malformation Terminology" was used [28].

Results

Approximately 5000 patients who visited our medical genetics clinic between 2012 and 2022 are recorded in our archive. Of these, 17 had a definitive genetic diagnosis. Patients' mutation forms and family trees in the NF1 gene are shown in Table 1. In addition, clinical, dysmorphic, and radiological imaging findings of the disease are also given in Table 2.

In this study, 5 out of 17 patients were women. According to the genealogical analysis of the patients, de novo mutation was detected in only two patients (Case 12 and Case 15). The families of the other 13 patients had affected family members. All of the mutations were referred to as heterozygous mutations. Cases 5 and 6 were uncles-nephews, Cases 10 and 11 were siblings, and Cases 12 and 13 were mother-son. Two patients had the intronic mutation. The non-coding mutation was present in Case 3. In Case 7, there was a splicing mutation. In the other 13 patients, the mutation was located in the exon region. Of the 17 patients in the study, 13 had pathogenic mutations according to the American College of Medical Genetics (ACMG) mutation classification. One patient had the "uncertain significance" variant. The age range of patients with a definitive diagnosis was between 2 and 48 years.

In the study, 17 NF1 patients presented primarily because of café au lait spots on their bodies. Apart from these spots, other presentation symptoms were as follows; neurofibromas, lisch nodules, disorders of neurodevelopments, unilateral hearing loss, ataxic gait, seizures, and short stature. In addition, the most common dysmorphic facial feature of these patients was both a long face and a deep eye. The second most common was both the long chin and the Cupid's Bow feature. The third was thick eyebrows.

In 4 out of 17 patients, hamartoma or hamartoma-like images were noted on brain MRI. In addition, a normal brain MRI was performed on 4 patients. When the researchers evaluated Case 2, there was a pontocerebellar arachnoid cyst. Therefore, the patient had unilateral hearing loss. This was the only Case in the study with a false sensory mutation. In addition, cerebellar hamartoma was present in Case 2, Case 7, and Case 13. However, there was no evidence of cerebellar hamartoma in patients. Optic nerve thickening prevailed only in Case 13.

In addition, when the researchers evaluated Case 15, the NF1 mutation caused mental retardation and autism in the patient. Case 16 presented to us with café au lait stains on her body. Head circumference and brain MRI were normal. In addition, the patient's sister and mother had similar findings, and optic glioma was additionally detected in the sister.

When the researchers evaluated Case 17, there were widespread cafe au lait stains on her body. When the researchers examined the brain MRI, the researchers was that there were calcification and atrophic lesions in the brain. The patient was also diagnosed with epilepsy. Her mother had an NF1 mutation. Similar spots were also observed in her sister.

Table 1. Genetic analysis results and heredity.

CASE ID	AGE AT TESTING (YEARS)	MUTATION(S)			ZYGOSITY	MODE OF TRANSMISSION
		ALTERATION	EXONI / INTRON NUMBER	TYPE		
1	37	NJF1 c.4537 CST p.Arg1513X	Exon 35	Nonsense	Heterozygous	Autosomal dominant
2	23	NJF1 c.2531 T>6 p.L844R	Exon 21	Misssense	Heterozygous	Autosomal dominant
3	27	NJF1 c.2990+5 GSA	Intron 22	Non-coding	Heterozygous	Autosomal dominant
4	40	NJF1 c.6955 CST p.Q2319*	Exon 47	Nonsense	Heterozygous	Autosomal dominant
5	10	NJF1 c.910 CST p.R304*	Exon 9	Nonsense	Heterozygous	Autosomal dominant
6	32	NJF1 c.910 CST p.R304*	Exon 9	Nonsense	Heterozygous	Autosomal dominant
7	29	NF1 c.1392+16>T	Intron 12	Splicing	Heterozygous	Autosomal dominant
8	48	NJF1 c.4084C>T p.R1362*	Exon 30	Nonsense	Heterozygous	Autosomal dominant
9	8	N El c.6772C>T p. R2258*	Exon 45	Nonsense	Heterozygous	Autosomal dominant
10	22	NF1 c.109_110delGA p.G1u37Alafs*29	Exon 2	Frameshift	Heterozygous	Autosomal dominant
11	16	NF1 c.109_110delGA p.G1u37Alafs*29	Exon 2	Frameshift	Heterozygous	Autosomal dominant
12	36	NF1 c.1541_1542delAG p.O.514Rfs*43	Exon 14	Frameshift	Heterozygous	De novo
13	6	NJF1 c.1541_1542de1AG p.0514Rfs*43	Exon 14	Frameshift	Heterozygous	Autosomal dominant
14	2	NJF1 c.3011_3011de1A p.N10041fs*8	Exon 23	Frameshift	Heterozygous	Autosomal dominant
15	7	NF1 c.499_502deITGIT p.C1671s*10			Heterozygous	Autosomal dominant
16	19	NJF1 c.5675de1A p.K1892fs*12	Exon 38		Heterozygous	Autosomal dominant
17	10				Heterozygous	Autosomal dominant

Table 2. Patient clinical information and findings.

CASE ID	PRESENTING SYMPTOMS	DYSMORPHIC FEATURES	MRI FINDINGS
1	Multiple cafe au lait spots, neurofibromas, lisch nodules	Prominent supraorbital ridges, cheekbones prominence, deeply set eyes, prominent antihelix stems, protruding ears, macrotia, low insertion columella	Nonspecific hyperintense signal in T2-FLAIR A sequences which is oval configuration measured as 7x5 mm in the right frontal white matter at the centrum semiovale level, L2-S1 vertebra perineural cyst
2	Multiple cafe au lait spots, neurofibromas, unilateral hearing loss	Long face, broad forehead, deeply set eyes, broad eyebrows, thick eyebrows, long palpebral fissures, prominent antitragus, long ears, narow nasal bridge, fullness paranasal tissue, deep philtrum, exaggerated Cupid's Bow	Pontocerebellar aracnoid cyst, mega cisterna magna, right cerebellar hamartoma
3	Multiple cafe au lait spots	Long face, cheekbones prominence, broad chin, deeply set eyes, narrow nasal ridge, deep philtrum, exaggerated Cupid's Bow, thin lower lip wermillon	N/A
4	Multiple cafe au lait spots, neurofibromas	Long face, malar flattening, prominent nasolabial fold, broad chin, deeply set eyes, thick eyebrows, telecanthus, enlarged nares, wide nasal base, wide nasal bridge, deep philtrum, exaggerated Cupid's Bow, thin lower lip wermillon	N/A
5	Multiple cafe au lait spots, seizure, neurodevelopmental delay, lisch nodules	Malar flattening, thick eyebrows, telecanthus, thick ala nasis, bulbose nose, long philtrum, thick lower lip vermillon, thick upper lip vermillon	Arachnoid cysts, cavum septum pellucidum et vergae
6	Multiple cafe au lait spots, neurofibromas	Long face, narrow face, prominence cheekbone, tall chin, thick eyebrows, low hanging columella, wide nasal base, thick upper lip vemillon, thick lower lip	N/A
7	Multiple cafe au lait spots, neurofibromas	Brachycephaly, frontal balding, long face, prominence cheekbones, long chin, deeply set eyes, hypotelorism, sparse eyebrow, prominent antitragus, thick ala nasi, low insertion columella, narrow nasal bridge, smooth philtrum	Cerebellar hamartoma, neurofibromas
8	Multiple cafe au lait spots, neurofibromas, sarcoma excision from arm	Long face, cheekbones prominence, malar flattening, broad chin, tall chin, deeply set eyes, downslanted palpebral fissures, high insertion columella, malaligned philtral ridges	Triceps muscle sarcoma, bladder mesencimal sarcoma

No.			
9	Multiple cafe au lait spots, ataxic gait	Malar prominence, deeply set eyes, sparse eyebrows, infraorbital creases, upslanted palpebral fissures, ptosis, thick ala nasi, wide nasal bridge, wide nasal ridge, deep philtrum, exaggerated Cupid's Bow	Hamartomas in superficial and deep white matter, periventricular white matter, left cerebellar hemisphere, corpus callosum, bilateral globus pallidus
10	Multiple cafe au lait spots, neurofibromas	Full cheeks, midface prominence, tall chin, downslanted palpebral fissures, wide nasal base, thick lower vermillon	N/A
11	Multiple cafe au lait spots, neurofibromas	Triangular face, full cheeks, midface prominence, pointed chin, downslanted palpebral fissures, wide nasal base, thick lower vermillon	N/A
12	Multiple cafe au lait spots	Broad chin, tall chin, smooth philtrum, thin lower lip vermilion	N/A
13	Multiple cafe au lait spots, short stature	Midface prominence, pointed chin, tall chin, wide spaced eyes, upslanted palpebral fissures, telecanthus, overfolded helix, narrow nasal ridge, exaggerated Cupid's Bow	Hamartomas in brain stem, cerebellar hemisphere and cerebral hemispheres, thickening of optic nerve
14	Multiple cafe au lait spots, developmental delay	Broad forehead, short chin, prominet antihelix stem, serpenginous antihelix stem, wide nasal base, wide mouth	N/A
15	Multiple cafe au lait spots, regressive autism	Macrocephaly, loss of speech	NF1 findings on the brain MRI
16	Multiple cafe au lait spots	NF1 mutations in her sister and mother	N/A
17	Multiple cafe au lait spots, epilepsy	NF1 mutations in her mother	Calcifications and atrophic lesions

Discussion

Genotype and phenotype results of NF1 patients were presented in this study. Ophthalmological symptoms in patients were also examined. This new generation of genetic disease diagnosis methods can be routinely used in clinical practice for medical geneticists.

De novo mutations, which are changes in a gene that occurs for the first time in a family member, are common in autosomal dominant disorders. NF1 is one of these diseases. Nearly half of all NF1 Cases occur as a de novo disease. In our study, de novo mutation was detected in one patient. In our other patients, autosomal dominant mutations were detected [28].

Mutation type is one of the elements used to classify mutations. For this reason, the type of mutation is an important step in the genotyping process. The rate of mutation types in the NF1 gene is reported to be between 21% and 38% for a nonsense mutation. According to ClinVar genetic database, 407 of 6254 variants are reported as nonsense [29,30]. The rate of variation in the intronic region of the NF1 gene, where the gene is not translated, is reported to be 43% to 20%. ClinVar genetic database showed an intronic mutation rate of about 12%. Considering that the variants causing NF1 disease may occur not only in exon regions but also in intronic regions, genetic analysis should be preferred. For this reason, it is recommended that methods of genetic analysis such as next-generation sequencing, which can detect changes in intronic regions, are chosen [31].

According to the ACMG criteria, variants identified by genetic analysis are classified into 5 classes. Class 2 is reported as "likely to be pathogenic" and class 1 is reported as "pathogenic". The disease is thought to be caused by variants in these two criteria groups. So far, 96 potentially pathogenic and 1753 pathogenic variants of the NF1 gene have been reported in the ClinVar database. In addition, over 2800 different pathogenic variants of the NF1 gene were identified in the University of Alabama cohort [32].

In the literature, the pathogenic mutation detection rate is between 89% and 96%. In this study, only 2 of the 17 NF1 analyses were "likely pathogenic", which is a new mutation, while the other 15 analyses resulted in "pathogenic" variants [33].

Doctors are becoming increasingly successful in diagnosing genetic diseases as technology advances. These include DeepGestalt (Face2Gene) technology that uses artificial intelligence. This has been reported to be successful in diagnosing between 86-91% [34]. Next-generation phenotyping (NGP) programs such as Face2Gene are recommended to be used in routine examinations, especially by medical genetics doctors and pediatricians. In addition, studies have shown that Face2Gene has a high success rate in patients with significant dysmorphic facial features [35].

The brain is responsible for controlling our entire body. That is why it is protected by a very dense and protected layer of bone called a cranium. Until the invention of MRI, it was not easy to detect morphological changes in the brain. Today, researchers can almost take a photograph of the brain with MRI [36]. Research has shown that NF1 disease also leads to some changes in the brain. One trial found that 6,5% of people with NF1 had a normal MRI. This was 35% in our study. This result illustrates the wide range in which NF1 can occur. In genetics, this is defined as variable expression. NF1 is one of the genetic diseases with high variable expression [37].

In a study conducted in Spain in 2019, arachnoid cysts were detected in brain MRI in 3 of 85 NF1 patients. In our study, arachnoid cysts were detected in 1, hamartomas in 3, and calcified areas in 1 of 17 patients [38]. In another study conducted in Türkiye, hamartomatous lesions in the central nervous system were found in 16 of 19 patients with NF1. As a result, NF1 cannot be diagnosed with brain MRI alone. Because no specific picture exists for the condition. However, as the disease causes lesions in the brain, an MRI of the brain is recommended for patients with NF1 [39].

When the researchers looked at the family tree of Case 16, it was determined that there were similar findings in her mother and sister. The sister of

the Case also identified optical glioma. Similarly, in the study that was conducted by Dr. Parkhurst et al., among the 708 patients diagnosed with NF1, 30 patients had optic glioma. The general findings of half of the patients are visual loss, proptosis and early adolescence. It also showed two-sided placement for 19 patients, right-hand side for seven patients, and left-hand placement for four patients. In these studies, many of the NF1 patients did not go to the doctor for eye control, and in NF1 children, they said that they developed asymptomatic optic glioma at a time as early as one year old. They emphasized that with annual eye checks and early puberty screening, optical gliomas can be diagnosed early [40].

In our study, two out of 17 patients had Lisch nodules: Case 1 and Case 5. When the researchers evaluated similar studies, Dr. Abaloun et al. presented NF1 patients at the age of 45. In the ophthalmological examination of the patients, he identified Lisch nodules and P+ visual acuity in both eyes of a patient in biomicroscopic examination [41]. Only one patient had autism other than the general NF1 clinical features. Dr. Garg et al. included 207 patients in their studies. According to the responses of 109; 32 patients had high levels of autism, 29 patients had no secondary autism, and 48 patients had no autism. Then, 23 people from the high-level autism group, 16 people from the middle-level autism group, and 16 people from the non-autism group were randomly selected and reviewed. Their analysis stressed that more research should be done for NF1, but still high levels of autism can be seen [42].

Conclusion

The diagnosis of diseases is one step ahead of the treatment of them. Because if the necessary diagnosis is not made, treatment of the disease is not possible. While this situation was more difficult in the past, nowadays, with the developing technology, diseases can be diagnosed more easily. In NF1 disease, more information can be obtained as a result of genetics, imaging, and examinations of other branches. For this reason, clinical and genetic departments should carry out joint studies in terms of early diagnosis, treatment and follow-up of hereditary diseases.

Conflict of interest

There is no conflict of interest to disclosure regarding this study.

References

1. Gutmann DH, Ferner RE, Listernick RH, Korf BR, Wolters PL, Johnson KJ. Neurofibromatosis type 1. Nat Rev Dis Prim. 2017;3:1-17. doi: 10.1038/nrdp.2017.4.

2. Boyd KP, Korf BR, Theos A. Neurofibromatosis type 1. J Am Acad Dermatol. 2009;61(1):1-14. doi: 10.1016/j.jaad.2008.12.051.

3. Yap Y-S, McPherson JR, Ong C-K, Rozen SG, Teh BT, Lee ASG, et al. The NF1 gene revisited - from bench to bedside. Oncotarget. 2014;5(15):5873-92. doi: 10.18632/oncotarget.2194.

4. Wallace MR, Marchuk DA, Andersen LB, et al. Type 1 neurofibromatosis gene: Identification of a large transcript disrupted in three NF1 patients. Science. 249(4965):181-6. doi: 10.1126/science.2134734.

5. Maruoka R, Takenouchi T, Torii C, Shimizu A, Misu K, Higasa K, et al. The use of next-generation sequencing in molecular diagnosis of neurofibromatosis type 1: A validation study. Genet Test Mol Biomarkers. 2014;18(11):722-35. doi: 10.1089/gtmb.2014.0109.

6. Woodward LM. Ocular manifestations

of phakomatoses. Int Ophthalmol Clin. 2014;54(3):105-10. doi: 10.1097/IIO.000000000000037.

7. Lubs M-LE, Bauer MS, Formas ME, Djokic B. Lisch nodules in neurofibromatosis type 1. N Engl J Med. 1991;324(18):1264-6. doi: 10.1056/NEJM199105023241807.

8. Boley S, Sloan JL, Pemov A, Stewart DR. A quantitative assessment of the burden and distribution of Lisch nodules in adults with neurofibromatosis type 1. Invest Ophthalmol Vis Sci. 2009;50(11):5035-43. doi: 10.1167/iovs.09-3650.

9. Weleber RG, Zonana J. Iris hamartomas (Lisch nodules) in a case of segmental neurofibromatosis. Am J Ophthalmol. 1983;96(6):740-3. doi: 10.1016/S0002-9394(14)71917-8.

10. Chernoff KA, Schaffer JV. Cutaneous and ocular manifestations of neurocutaneous syndromes. Clin Dermatol. 2016;34(2):183-204. doi: 10.1016/j.clindermatol.2015.11.003.

11. Abdolrahimzadeh S, Plateroti AM, Recupero SM, Lambiase A. An update on the ophthalmologic features in the phakomatoses. J Ophthalmol. 2016:1-15. doi: 10.1155/2016/3043026.

12. Kinori M, Hodgson N, Zeid JL. Ophthalmic manifestations in neurofibromatosis type 1. Surv Ophthalmol. 2018;63(4):518-33. doi: 10.1016/j.survophthal.2017.10.007.

13. Laue L, Comite F, Hench K, Loriaux DL, Cutler GB, Pescovitz OH. Precocious puberty associated with neurofibromatosis and optic gliomas: Treatment with luteinizing hormone releasing hormone analogue. Am J Dis Child. 1985;139(11):1097-100. doi: 10.1001/archpedi.1985.02140130035025.

14. Listernick R, Ferner RE, Liu GT, Gutmann DH. Optic pathway gliomas in neurofibromatosis-1: Controversies and recommendations. Ann Neurol. 2007;61(3):189-98. doi: 10.1002/ana.21107.

15. Cassiman C, Legius E, Spileers W, Casteels I. Ophthalmological assessment of children with neurofibromatosis type 1. Eur J Pediatr.

2013;172(10):1327-33. doi: 10.1007/s00431-013-2035-2.

16. Packer RJ, Ater J, Allen J, Phillips P, Geyer R, Nicholson HS, et al. Carboplatin and vincristine chemotherapy for children with newly diagnosed progressive low-grade gliomas. J Neurosurg. 1997;86(5):747-54. doi: 10.3171/jns.1997.86.5.0747.

17. Kestle JRW, Hoffman HJ, Mock AR. Moyamoya phenomenon after radiation for optic glioma. J Neurosurg. 1993;79(1):32-5.

18. Ferner RE, Huson SM, Thomas N, Moss C, Willshaw H, Evans DG, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. J Med Genet. 2007;44(2):81-8. doi: 10.1136/jmg.2006.045906.

19. Dasgupta B, Yi Y, Chen DY, Weber JD, Gutmann DH. Proteomic analysis reveals hyperactivation of the mammalian target of rapamycin pathway in neurofibromatosis 1-associated human and mouse brain tumors. Cancer Res. 2005;65(7):2755-60. doi: 10.1158/0008-5472.CAN-04-4058.

20. Weiss B, Widemann BC, Wolters P, Dombi E, Vinks A, Cantor A, et al. Sirolimus for progressive neurofibromatosis type 1–associated plexiform neurofibromas: A Neurofibromatosis Clinical Trials Consortium phase II study. Neuro Oncol. 2015;17(4):596-603. doi: 10.1093/neuonc/nou235.

21. Robertson KA, Nalepa G, Yang F-C, Bowers DC, Ho CY, Hutchins GD, et al. Imatinib mesylate for plexiform neurofibromas in patients with neurofibromatosis type 1: A phase 2 trial. Lancet Oncol. 2012;13(12):1218-24. doi: 10.1016/S1470-2045(12)70414-X.

22. Ober RR, Palmer EA, Drack A V, Wright KW. Retinopathy of prematurity. Handb Pediatr Retin Dis. 2006; 284-349 p.

23. Grant WM, Walton DS. Distinctive gonioscopic findings in glaucoma due to neurofibromatosis. Arch Ophthalmol. 1968;79(2):127-34. doi: 10.1001/archopht.1968.03850040129003.

24. Javadi MA, Kanavi MR, Faramarzi A, Feizi S, Azizi F, Javadi F. Confocal scan imaging and impression cytology of the cornea in a case of multiple endocrine neoplasia type-2b. J Ophthalmic Vis Res. 2012;7(2):176-9.

25. Shaheen BS, Bakir M, Jain S. Corneal nerves in health and disease. Surv Ophthalmol. 2014;59(3):263-85. doi: 10.1016/j.survophthal.2013.09.002.

26. Rescaldani C, Nicolini P, Fatigati G, Bottoni FG. Clinical application of digital indocyanine green angiography in choroidal neurofibromatosis. Ophthalmologica. 1998;212(2):99-104. doi: 10.1159/000027287.

27. Destro M, D'Amico DJ, Gragoudas ES, Brockhurst RJ, Pinnolis MK, Albert DM, et al. Retinal manifestations of neurofibromatosis: Diagnosis and management. Arch Ophthalmol. 1991;109(5):662-6. doi: 10.1001/archopht.1991.01080050076033.

28. Nussbaum RL, McInnes RR, Willard HF. Thompson & Thompson Genetics in Medicine E-Book. Elsevier Health Sciences; 2015.

29. Zhang J, Tong H, Fu X, Zhang Y, Liu J, Cheng R, et al. Molecular characterization of NF1 and neurofibromatosis type 1 genotype-phenotype correlations in a Chinese population. Sci Rep. 2015;5(1):11291. doi: 10.1038/srep11291.

30. Thomson SAM, Fishbein L, Wallace MR. NF1 mutations and molecular testing. J Child Neurol. 2002;17(8):555-61. doi: 10.1177/088307380201700803.

31. Pros E, Gómez C, Martín T, Fábregas P, Serra E, Lázaro C. Nature and mRNA effect of 282 different NF1 point mutations: Focus on splicing alterations. Hum Mutat. 2008;29(9):173-93. doi: 10.1002/humu.20826.

32. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24. doi: 10.1038/gim.2015.30.

33. Evans DG, Bowers N, Burkitt-Wright E, Miles E, Garg S, Scott-Kitching V, et al. Comprehensive RNA analysis of the NF1 gene in classically affected NF1 affected individuals meeting NIH criteria has high sensitivity and mutation negative testing is reassuring in isolated Cases with pigmentary features only. EBioMedicine. 2016;7:212-20. doi: 10.1016/j.ebiom.2016.04.005.

34. Gurovich Y, Hanani Y, Bar O, Nadav G, Fleischer N, Gelbman D, et al. Identifying facial phenotypes of genetic disorders using deep learning. Nat Med. 2019;25(1):60-4. doi: 10.1038/s41591-018-0279-0.

35. Mishima H, Suzuki H, Doi M, Miyazaki M, Watanabe S, Matsumoto T, et al. Evaluation of Face2Gene using facial images of patients with congenital dysmorphic syndromes recruited in Japan. J Hum Genet. 2019;64(8):789-94. doi: 10.1038/s10038-019-0619-z.

36. Rosenbaum T, Engelbrecht V, Krölls W, van Dorsten FA, Hoehn-Berlage M, Lenard HG. MRI abnormalities in neurofibromatosis type 1 (NF1): A study of men and mice. Brain Dev. 1999;21(4):268-73. doi: 10.1016/s0387-7604(99)00024-8.

37. Sabbagh A, Pasmant E, Laurendeau I, Parfait B, Barbarot S, Guillot B, et al. Unravelling the genetic basis of variable clinical expression in neurofibromatosis 1. Hum Mol Genet. 2009;18(15):2768-78. doi: 10.1093/hmg/ddp212.

38. Marco SBS, Pisón JL, Escribano CC, Viejo IG, Gallart MDM, Villagrasa PS. Neurological manifestations of neurofibromatosis type 1: Our experience. Neurol (English Ed). 2022;37(5):325-33. doi: 10.1016/j.nrleng.2019.05.008.

39. Keleşoğlu KS, Keskin S, Sivri M, Erdoğan H, Nayman A, Koplay M. Neurofibromatosis type 1: Cranial MRI findings [in Turkish]. Genel Tıp Derg. 2014;24:150-4.

40. Parkhurst E, Abboy S. Optic gliomas in neurofibromatosis type 1. J Pediatr

Ophthalmol Strabismus. 2016;53(6):334-8. doi: 10.3928/01913913-20160810-03.

41. Abaloun Y, Ajhoun Y. Lisch nodule in neurofibromatosis type 1. Pan Afr Med J. 2017;27:218. doi:10.11604/pamj.2017.27.218.11517.

42. Garg S, Green J, Leadbitter K, Emsley R, Lehtonen A, Evans DG, et al. Neurofibromatosis type 1 and autism spectrum disorder. Pediatrics. 2013;132(6):1642-8. doi: 10.1542/peds.2013-1868.