

**Case Report**

Case Series: Neurofibromatosis Type 1 in 8 Pediatric Patients; Genotype and Phenotype Analysis

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Abstract: Neurofibromatosis type I is a multi-systemic disorder caused by variants in the neurofibromin, a gene on chromosome 17 that regulates a variety of cellular functions important for tumorigenesis. Clinically it is characterized by multiple café-au-lait spots, intertriginous freckling, neurofibromas, and learning disability or behavior problems. Neurofibromatosis type I is inherited in an autosomal dominant manner, but approximately half of those afflicted have the condition as a result of a de novo disease-causing variant. In this case series, we present 8 pediatric patients at Mother Teresa University Hospital Center in Tirana, Albania whose genetic diagnosis was confirmed by Whole exome sequencing including Next-generation sequencing-based Copy number variation analysis. Based on our clinical findings, they all meet the revised clinical diagnostic criteria of Neurofibromatosis type I by the International Consensus Conference held in 2021. In 5 of them, the variant was inherited from one of the parents, while in the remaining 3 the variant was de novo. Herein we discuss each genotype detected, the coordinates, class, and the modifications they induce in the neurofibromin protein. We also discuss our patients' phenotypes; the cases of two pairs of siblings with identical inherited variants but with different clinical manifestations caught our interest, but studies confirm that the phenotype varies even among individuals with identical variants. In the end, identifying the signs and symptoms early and with certainty and rapidly assigning the cases to qualified healthcare professionals is important for patients with neurofibromatosis type I.

Keywords: Neurofibromatosis 1, NF1, Rare Diseases, Pediatric Patients

1. Introduction

Neurofibromatosis type I (NF1) is a multi-system disorder caused by variants in neurofibromin, a gene on chromosome 17 that codes a homonymous GTPase-activating protein that negatively regulates RAS/RAF/MAPK pathway activity by accelerating the hydrolysis of Ras-bound GTP [1-4]. This complex pathway transduces signals from the extracellular environment to the cell nucleus, regulating a variety of cellular functions important for tumorigenesis such as cell growth, proliferation, differentiation, cell migration, as well as angiogenesis [5, 6].

NF1 is inherited in an autosomal dominant manner, but studies say that approximately 50% of those afflicted have the condition due to a de novo disease-causing variant [7]. The incidence at birth is around 1:2600 to 1:3000 individuals [8].

2. Case Presentations

This study enrolled 8 pediatric patients with variants in the NF1 gene at Mother Teresa University Hospital Center in Tirana, Albania. The genetic diagnosis of autosomal dominant neurofibromatosis type 1 is confirmed by Whole Exome

Sequencing (WES) including NGS-based CNV analysis. Patients 1 and 2 as well as patients 3 and 4 are siblings. Variant coordinates are after the Human Genome Variation Society (HGVS) nomenclature as provided by the laboratory reports. The classes of the variants are defined according to the standards and guidelines for the interpretation of sequence variants of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP). The clinical information (phenotype) indicated below follows the Human Phenotype Ontology (HPO) nomenclature. Table 1 presents the summary of our patients' genetic data.

Three of our eight patients had a *de novo* variant discovered.

Patient 1

Patient 1 is a 14-year-old girl that presented café-au-lait spots, seizure, and scoliosis. The brain magnetic resonance imaging (MRI) was without significant findings. The age at onset was 22 months. Regarding the family history, the mother had café-au-lait spots and neurofibromas. Genetic testing identified a heterozygous, pathogenic (class 1), maternally inherited variant in the NF1 gene with coordinates c.4084C>T.

Patient 2

Patient 2 is a 12-year-old girl and is the sibling of patient 1, with whom she shares the same variant inherited from the mother. She presented only multiple café-au-lait spots.

Patient 3

Patient 3 is an 11-year-old girl that presented encephalopathy, multiple café-au-lait spots, and neurofibroma. The age at onset was 8 months. Regarding the family history, the father had café-au-lait spots and neurofibromas. Genetic testing identified a heterozygous, likely pathogenic (class 2), paternally inherited variant in the NF1 gene with coordinates c.573dup.

Patient 4

Patient 4 is an 8-year-old boy and is the brother of patient 3,

with whom he shares the same variant inherited from the father. He presented abnormality of the eye and face, multiple café-au-lait spots, cutaneous neurofibromas, and a plexiform neurofibroma. The brain MRI was normal. Genetic testing identified the same variant as his father and sister.

Patient 5

Patient 5 is a 10-year-old boy that presented with café-au-lait spots. The electroencephalography (EEG) was normal. The age at onset was 3 years. Regarding the family history, there was nothing to be noted. Genetic testing identified a heterozygous pathogenic (class 1) variant in the NF1 gene with coordinates c.1007G>A. *De novo* status has been confirmed by parental testing.

Patient 6

Patient 6 is a 9-year-old boy that presented café-au-lait spots, headache, hyperreflexia, hypertonia, intention tremor, and macrocephaly. Regarding the family history, the father had neurofibromas. Genetic testing identified a heterozygous, likely pathogenic, paternally inherited variant in the NF1 gene with coordinates c.1393-1G>A.

Patient 7

Patient 7 is an 8-year-old girl that presented abnormality of the face, brain atrophy, cerebral palsy, delayed speech and language development, encephalopathy, hyperreflexia, intellectual disability, macrocephaly, motor delay, seizure, and spasticity. Regarding the family history, there was nothing to be noted. Genetic testing identified a heterozygous, likely pathogenic variant in the NF1 gene with coordinates c.3473A>T. *De novo* status was confirmed by parental testing.

Patient 8

Patient 8 is a 3-year-old boy that presented multiple café-au-lait spots. The psycho-motor development was normal; MRI was also without significant findings. The age at onset was 8 months. Genetic testing identified a heterozygous, likely pathogenic variant in the NF1 gene with coordinates c.5878dup. *De novo* status has been confirmed by parental testing.

Table 1. The summary of our patients' genetic data.

No./Sex/	Variant coordinates	Amino acid change	Variant type	Variant status	Disease-associated mechanism	Class. ACMG
1/ F	c.4084C>T	p.(Arg1362*)	substitution	maternally inherited	nonsense	class 1
2/ F	c.4084C>T	p.(Arg1362*)	substitution	maternally inherited	nonsense	class 1
3/ F	c.573dup	p.(Arg192Thrfs*9)	duplication	paternally inherited	frameshift	class 2
4/ M	c.573dup	p.(Arg192Thrfs*9)	duplication	paternally inherited	frameshift	class 2
5/ M	c.1007G>A	p.(Trp336*)	substitution	<i>de novo</i>	nonsense	class 1
6/ M	c.1393-1G>A	-	splice-site	paternally inherited	splicing	class 2
7/ M/	c.3473A>T	p.(Asp1158Val)	substitution	<i>de novo</i>	missense	class 2
8/ M	c.5878dup	p.(Cys1960Leufs*4)	duplication	<i>de novo</i>	frameshift	class 2

Class. ACMG, classification according to the American College of Medical Genetics and Genomics; F, female; M, male; No., number; -, unknown

3. Discussion

Many advances in diagnosis and treatment have been achieved since 1882, the year when NF1 was first described

by Friedrich Daniel von Recklinghausen [9]. According to the International Consensus Conference held in 2021, NF1 diagnosis is based on clinical findings as well as genetic test results, as shown in Table 2 [10].

Table 2. Revised diagnostic criteria of NF1 from the International Consensus Conference, 2021.

1. An individual who does not have a parent diagnosed with NF1, if two or more of the following are present:
a) six or more café-au-lait macules > 5 mm in greatest diameter in prepubertal individuals and > 15 mm in greatest diameter in postpubertal individuals ^a
b) freckling in the axillary or inguinal region ^a
c) two or more neurofibromas of any type or one plexiform neurofibroma
d) optic pathway glioma
e) two or more iris Lisch nodules or two or more choroidal abnormalities (CAs*)
f) a distinctive osseous lesion such as sphenoid dysplasia ^b , anterolateral bowing of the tibia, or pseudarthrosis of a long bone
g) a germline NF1 pathogenic variant
2. A child of a parent who meets the diagnostic criteria specified in [1], if one or more of the criteria in [1] are present.

a) If only café-au-lait macules and freckling are present, the diagnosis is most likely NF1 but other diagnoses are possible; at least one of these two findings should be bilateral.

b) Sphenoid wing dysplasia is not a separate criterion in the case of an ipsilateral orbital plexiform neurofibroma.

* defined as bright, patchy nodules imaged by optical coherence tomography or near-infrared reflectance imaging

Genotype

Patients 1 and 2 are siblings and their variant is a substitution that creates a premature stop codon, consequently, its disease-associated mechanism is nonsense. The predicted amino acid change is p. (Arg1362*). This variant was previously described by Upadhyaya et al., 1997, Xiong et al., 2015, and Tsipi et al., 2018 [11-13].

Patients 3 and 4 are also siblings, their variant is a duplication that creates a shift in the reading frame starting at codon 192 and the new reading frame ends in a stop codon 8 positions downstream. The predicted amino acid change is p.(Arg192Thrfs*9).

Patient 5's variant is a substitution and creates a premature stop codon, thus its disease-causing mechanism is nonsense. The predicted amino acid change is p.(Trp336*). This variant is previously described by Wimmer et al., 2000, and Zatkova et al., 2004 [14, 15].

Patient 6's variant is predicted to disrupt the highly conserved acceptor splice site, thus its disease-causing mechanism is splicing.

Patient 7's variant is a substitution, its disease-causing mechanism is missense and the predicted amino acid change is p.(Asp1158Val).

Patient 8's variant is a duplication, it creates a shift in the reading frame starting at codon 1960, and the new reading frame ends in a stop codon 3 positions downstream. The predicted amino acid change is p.(Cys1960Leufs*4).

Phenotype

NF1 is clinically characterized by multiple café-au-lait spots, intertriginous freckling, neurofibromas, and learning disability or behavior problems. Peripheral nerve sheath tumors, optic nerve and other central nervous system gliomas, scoliosis, bone dysplasia, vasculopathy, and gastrointestinal, endocrine, or pulmonary disorders are less frequent but more severe symptoms [7].

The case of our siblings who, despite having the same variant, had different clinical presentations, piqued our interest. According to genotype-phenotype correlation studies, identifying a specific NF1 variation even within a family cannot predict the course or prognosis of the disease in a patient with NF1; however, only four clinically confirmed correlations have been reported: (i) NF1 p.Met992del, (ii) NF1 p.Arg1809, (iii) NF1 microdeletion, and (iv) missense

mutations affecting 1 of the 5 codons 844–848 [11]. Neither of our patients had one of these variants.

To conclude, patients 1, 2, 3, 4, and 6 are children of a parent who meets the diagnostic criteria, and present at least one criterion shown in [1] in Table 2. Patients 5, 7, and 8 had de novo variants, and presented at least two of the criteria shown in [1] in Table 2. Therefore, the diagnosis of Neurofibromatosis type 1 is confirmed in all of our eight patients.

4. Conclusion

We present a case series of eight pediatric patients who meet the diagnostic criteria of Neurofibromatosis type 1. NF1 is a complex rare genetic disease manifested usually during childhood in a variety of clinical aspects even between individuals with the same variant in the NF1 gene. Recognizing the typical characteristic signs and symptoms of NF1 patients, identifying them early, and rapidly assigning them to qualified healthcare professionals is crucial.

Conflict of Interest

The authors declare that they have no competing interests.

Authors' Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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