

RESEARCH

Open Access



Whole-exome sequencing revealed a likely pathogenic variant in *NF1* causing neurofibromatosis type I and Arrhythmogenic Cardiomyopathy

Maryam Pourirahim¹, Golnaz Houshmand², Leyla Abdolkarimi², Majid Maleki¹ and Samira Kalayinia^{1*}

Abstract

Background Neurofibromatosis type I (NF1) is a genetic disorder characterized by the tumor's development in nerve tissue. Complications of NF1 can include pigmented lesions, skin neurofibromas, and heart problems such as cardiomyopathy. In this study, we performed whole-exome sequencing (WES) on an Iranian patient with NF1 to identify the genetic cause of the disease.

Methods Following clinical assessment, WES was used to identify genetic variants in a family with a son suffering from NF1. No symptomatic manifestations were observed in other family members. In the studied family, *in silico* and segregation analysis were applied to survey candidate variants.

Results Clinical manifestations were consistent with arrhythmogenic cardiomyopathy (ACM). WES detected a likely pathogenic heterozygous missense variant, c.3277G > A:p.Val1093Met, in the *NF1* gene, confirmed by PCR and Sanger sequencing. The patient's parents and brother had a normal sequence at this locus.

Conclusions Although there is no cure for NF1, genetic tests, such as WES, can detect at-risk asymptomatic family members. Furthermore, cardiac evaluation could also help these patients before heart disease development.

Keywords Neurofibromatosis, Genetic, Variant, Cardiomyopathy, Arrhythmogenic cardiomyopathy

Introduction

Neurofibromatosis (NF), with an incidence of 1 to 5 per 10 000 live births worldwide, is a genetic disorder that causes tumors in nerve tissue [1, 2]. The inheritance pattern of NF is autosomal dominant [2] and is divided into 3 categories: NF1, NF2, and schwannomatosis [3]. NF1,

also called “von Recklinghausen’s disease”, is caused by germ-line mutations in tumor suppressor genes [4], with symptoms including pigmentary lesions (brown macules, freckles, skin folds, and Lisch nodules), skin neurofibromas, skeletal abnormalities (scoliosis, tibia pseudarthrosis, and orbital dysplasia), brain tumors (gliomas of the visual pathway and glioblastomas), and peripheral nerve tumors (spinal neurofibromas, plexiform neurofibromas, and malignant peripheral nerve sheath tumors) [4]. It typically manifests during childhood, although it can appear in adulthood as well. NF1 sometimes causes cardiovascular diseases [5], such as congenital heart diseases [6] and cardiomyopathies [7]. In a study by Ibarrola et al.,

*Correspondence:

Samira Kalayinia
samira.kalayi@yahoo.com

¹Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran

²Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

left ventricular non-compaction and orthodromic atrio-ventricular tachycardia were observed in a patient with NF1 [8]. Moreover, a patient with NF1 and hypertrophic cardiomyopathy (HCM) was described by Jurko et al. [9]. Nguyen et al. [10] concluded that in patients with sizeable deletions of the *NF1* gene, HCM was more common. *NF1* gene, located on chromosome 17q11.2 and encodes a large cytoplasmic protein known as neurofibromin. Neurofibromin serves as a tumor suppressor and plays a critical role in the regulation of cellular signaling pathways, particularly the Ras/mitogen-activated protein kinase (MAPK) pathway. Treatment for NF1 focuses on managing symptoms and complications rather than curing the condition. In the present study, we performed whole-exome sequencing (WES) on an Iranian family with a son suffering from NF1 and arrhythmogenic cardiomyopathy (ACM) to identify the genetic cause(s). ACM is a genetic disorder characterized by the replacement of heart muscle tissue with fibrous or fatty tissue. It typically manifests in adolescence or young adulthood,

although it can present at any age. Arrhythmias and chest pain are the common features of ACM. Genetic counseling and testing for affected NF1 and/or ACM individuals and their families to identify the mutation and assess the risk of passing it on to future generations. To our knowledge, this is the first report of ACM manifestation in a patient with NF1.

Methods

Study subject, clinical evaluation, and ethics statement

A 20-year-old male patient was referred to the Cardio-genetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran with a complaint of chest pain and arrhythmias. The patient’s cardiac symptoms started at 16 years old, and dark spots were observed on his body at 2 years old. He was the second child of non-consanguineous healthy parents (Fig. 1A). A comprehensive clinical workup, including echocardiography, magnetic resonance imaging, and physical examination,

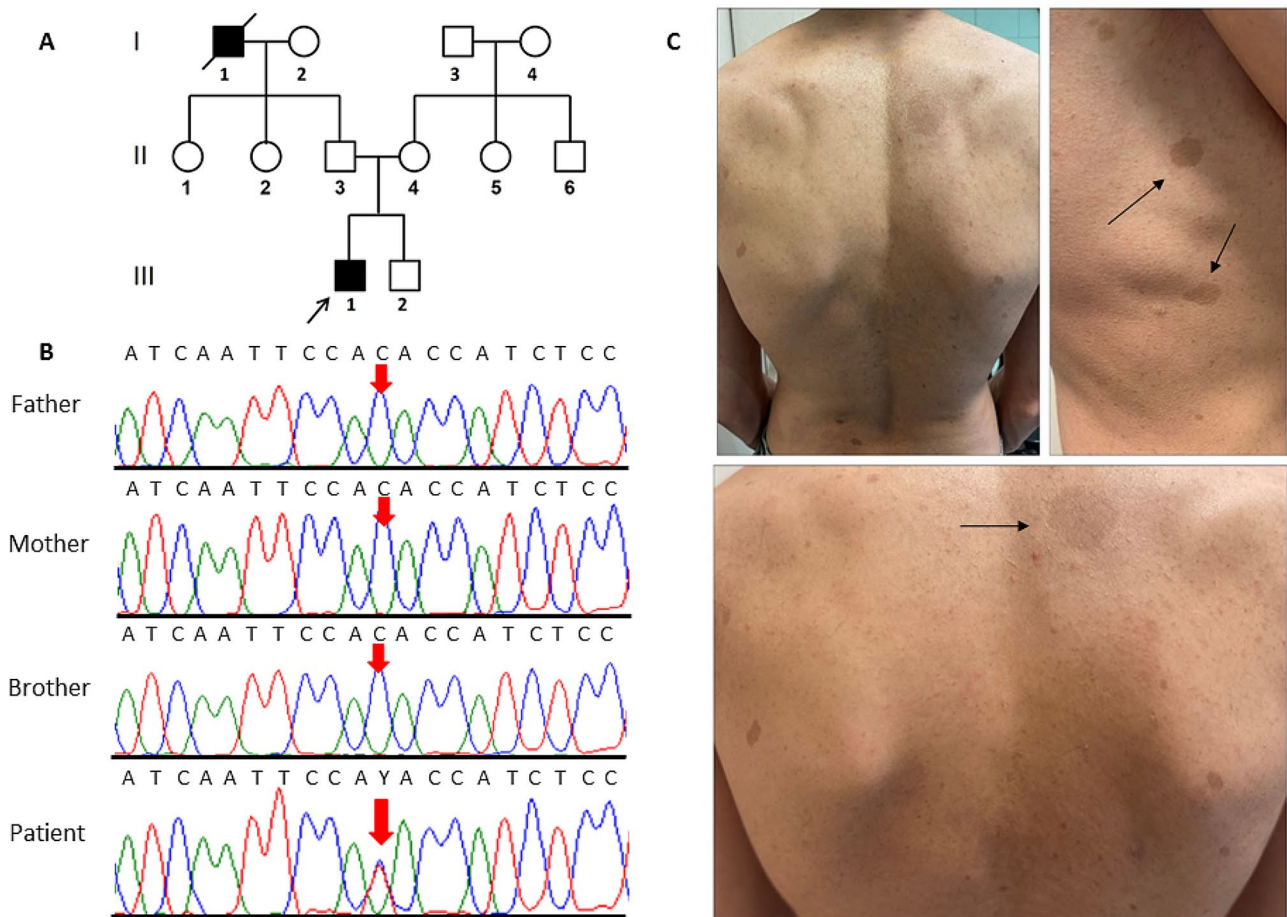


Fig. 1 The image presents pedigree, sequencing chromatograms, and neurofibromatosis type 1 clinical manifestations in a family affected by *NF1* mutation. **(A)** The pedigree of an Iranian family with NF1. Only the proband (III-1) is affected (pointed with an arrow). **(B)** The snapshot of the sequencing reads: The proband (III-1) carries the c.3277G>A variant in a heterozygous status. The black arrow shows the location of the mutated nucleotide. **(C)** Clinical manifestations of the proband (III-1) with NF1 which includes multiple brown spots in different parts of the patient’s body

was performed. The results of the clinical investigations of the other family members were normal. For precise diagnosis, WES was done on the proband (Fig. 1A: III-2). The study was performed in accordance with the Helsinki Declaration and was approved by the Ethics Committee of Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran (IR.RHC.REC.1402.017). Written informed consent was obtained from the family.

WES and segregation analysis

Genomic DNA was extracted from the peripheral blood samples of the proband and his family members using the Roche kit (DNsol Mini kit of Roche: lot No. 500,021,091,210,050). WES was performed at Macrogen (Amsterdam, Netherlands) on the Illumina HiSeq 6000. Raw data were analyzed by Rajaie Cardiovascular Medical and Research Center, Tehran, Iran. BWA, v.07.17, was used to align the reads to the reference genome (NCBI, hg19) [11]. Single-nucleotide polymorphisms and insertions and/or deletions were called using GATK, v.4.1.4.1 [12]. All the determined variants were annotated by ANNOVAR [13]. Frequency databases, consisting of Iranome (<http://www.iranome.ir>), Greater Middle East (<http://igm.ucsd.edu/gme/>), 1000 Genomes Project (<http://www.1000genomes.org>), and Genome Aggregation Database (<https://gnomad.broadinstitute.org>), were drawn upon to filter the variants. The variants with a frequency of more than 1% in these databases were excluded. In the next step, synonymous, intronic, and intergenic variants were removed. Then, variants related to genes associated with the patient's phenotype were retained. The pathogenicity of the variants was evaluated using prediction tools, composed of CADD (<https://cadd.gs.washington.edu/home>), SIFT (<https://sift.bii.a-star.edu.sg/>), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), PROVEAN (<http://provean.jcvi.org/index.php>),

and MutationTaster (<http://www.mutationtaster.org/>). The results were interpreted based on the 2015 guidelines of the American College of Medical Genetics and Genomics (ACMG) [14]. The conservation analysis of the protein change position was performed by comparing the amino acid sequences of different species on PolyPhen-2.

Segregation analysis was carried out by polymerase chain reaction (PCR)-direct Sanger sequencing to evaluate the family members. For variant amplification, Primer F (5'-AGGTTTATTTGAGGGGAAGTG-3') and Primer R (5'-ACTCAATGCCAACGTAGACA G-3') were designed using Gene Runner, v. 6.5.52. PCR was performed (the denaturing stage: 30 s at 95 °C, the annealing stage: 30 s at 60 °C, and the extending stage: 30 s at 72 °C [35 cycles]) on a SimpliAmp (Thermo Fisher Scientific) with 100 ng DNA, 1.5 mmol/L of MgCl₂, 200 mmol/L of dNTP, 10 pmol/L of the primers, and 1 U of Taq DNA polymerase (Amplicon, UK). The PCR products were sequenced with the ABI Sequencer 3500XL PE machine (Applied Biosystems) and analyzed using BioEdit, v.7.0.5.3.

Results

Clinical evaluation

The physical examination of the proband (Fig. 1A: III-1) indicated poor eyesight and more than 6 flat and light brown spots (café-au-lait spots) on the skin of his legs, hands, back, chest, sides, armpits, neck, and abdomen (Fig. 1C). The patient had learning disability, especially with mathematics and reading. According to the description given by the family, hyperactivity disorder and speech delays were also observed in the patient's childhood. Magnetic resonance imaging (Fig. 2A-C) and echocardiography (Fig. 3A-D) revealed right ventricular ACM. Cardiac magnetic resonance imaging showed a normal left ventricular size, a left ventricular end-diastolic volume indexed to the body surface area of 74

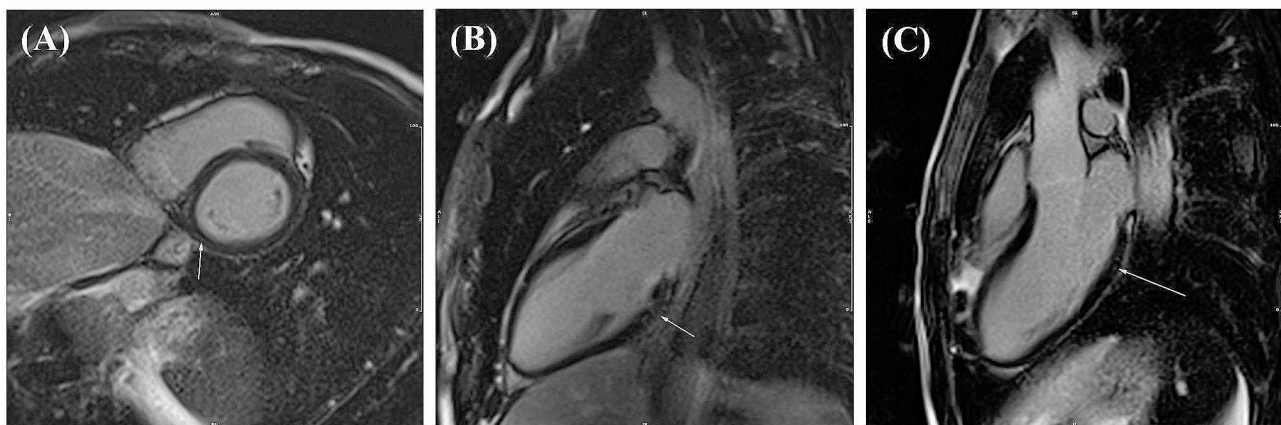


Fig. 2 The late enhancement images at the basal level short-axis view and two-and three-chamber views, respectively, show subepicardial enhancement in the basal inferior, inferolateral walls of the left ventricle

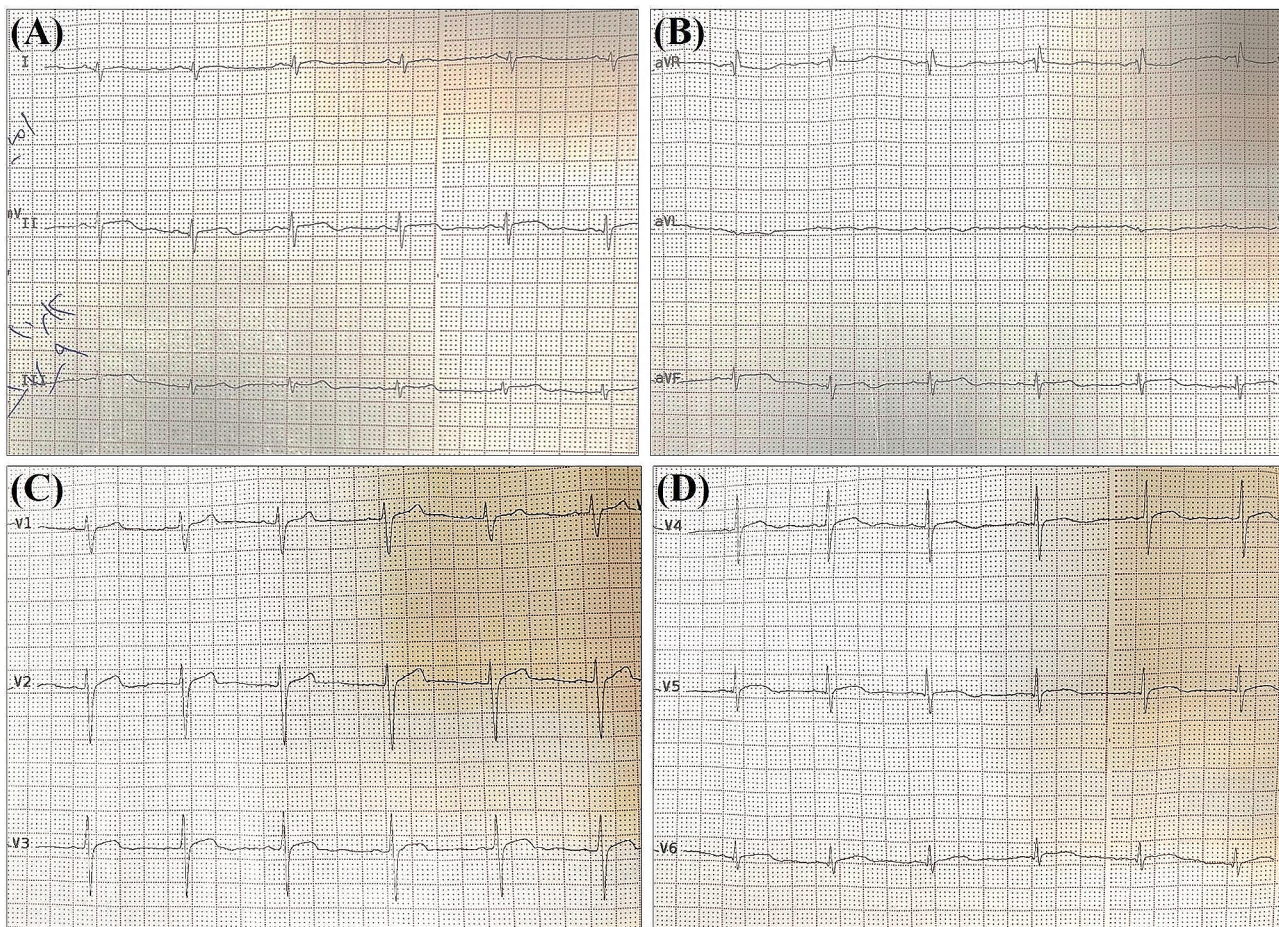


Fig. 3 The 12-lead ECG showing rS in the inferior leads and persistent, prolonged S and poor R progression in the precordial leads. Right ventricular hypertrophy (RVH) was seen in the ECG

mL/m², a moderately reduced left ventricular ejection fraction of 43%, a normal right ventricular size, an end-diastolic volume indexed to the body surface area of 65 mL/m², and a mildly reduced right ventricular ejection fraction of 42%. Segmental dyskinesia was observed in the sub-tricuspid, mid-right ventricular free wall, and right ventricular outflow regions (Supplementary Videos 1–3). The short tau inversion recovery (STIR) sequences indicated no inflammation or edema. Late gadolinium enhancement sequences showed subepicardial fibrosis in the basal inferior and inferolateral walls of the left ventricle (Fig. 2). According to the cardiac magnetic resonance imaging features, consisting of biventricular systolic dysfunction, right ventricular segmental dyskinesia, and fibrotic patterns in the left ventricular myocardium, the phenotype was considered compatible with ACM with biventricular involvement.

Molecular evaluation

WES detected a heterozygous variant in exon 25 of the *NF1* gene (NM_001042492.3: c.3277G>A:p.Val1093Met)

in the proband (Fig. 1A: III-1), probably responsible for neurofibromatosis type I (NF1). Based on the ACMG classification (PM1, PM2, PM5, and PP5), c.3277G>A is a likely pathogenic variant. PCR and Sanger sequencing confirmed the c.3277G>A variant in the affected proband. The patient's unaffected parents (Fig. 1A: II-3 and II-4) and brother (Fig. 1A: III-2) were normal for this locus (Fig. 1B). The missense variant was supported as the cause of the disease by CADD, SIFT, PolyPhen-2, PROVEAN, FATHMM, and GERP⁺⁺ (Table 1).

Discussion

In our present study, we found a missense variant, c.3277G>A, in exon 25 of the *NF1* gene in the proband, probably responsible for his NF1 condition. To our knowledge, our investigation is the first to report the association between the *NF1* variant and ACM. Previous studies have reported other cardiovascular manifestations secondary to *NF1* mutations, including congenital heart diseases, vasculopathy, and hypertension [15]. Dunning-Davies et al. [16] concluded that

Table 1 Identified variant in this study

Gene/transcript	Variant	¹ CADD	² SIFT	³ PolyPhen-2	⁴ PROVEAN	⁵ FATHMM	⁶ GERP++
<i>NF1</i>	c.3277G>A	28.7	0.04	0.9	-2.8	0.9	5.3
NM_001042492.3	p.Val1093Met						

¹ CADD, Phred \leq 20: Natural; Phred $>$ 20: Damaging

² SIFT, score \leq 0.05: Deleterious; score $>$ 0.05: Tolerable

³ PolyPhen-2, score \leq 0.15: Benign; score $>$ 0.85: Damaging

⁴ PROVEAN, score \leq -2.5: Deleterious; score $>$ -2.5: Tolerable

⁵ FATHMM, score \leq 0.5: Neutral; score $>$ 0.5: Deleterious

⁶ GERP++ scores range from -12.3 to 6.17, higher scores indicating conserve locus.

congenital heart diseases, especially pulmonary stenosis, were associated with, with frequencies ranging from 0.4 to 6.4%. *NF1*-associated vasculopathy, comprises stenosis, aneurysms, and arteriovenous malformations, the second cause of death worldwide, renal artery stenosis is the most common appearance, occurring in at least 1% of patients with *NF1*. Nonetheless, the clinical investigation of the proband did not detect vasculopathy. Hypertension is strongly associated with increased mortality in *NF1* patients and should be screened annually [15]. Prior to our current study, hypertension was not observed in similar investigations, although this complication may occur in older age. In 2019, Ritter et al. [17] described a patient with isolated fetal HCM as the presenting feature of *NF1*. Left ventricular hypertrophy, a rare finding in *NF1*, is primarily reported in patients with 1.4 Mb *NF1* deletion. The loss of neurofibromin in cardiomyocytes causes cardiac hypertrophy and progressive HCM in *NF1* homozygous null mouse models, suggesting that adults with *NF1* who have a normal heart structure in childhood might be at risk of cardiomyopathy later in life. Most likely, fetal cardiomyopathy, asymmetric septal hypertrophy, and subsequent diagnosis of *NF1* are not coincidental. Nevertheless, given that the patient's cardiac dysfunction in the study by Ritter et al. had no other causes, the most likely explanation is that cardiomyopathy was an early manifestation of left ventricular hypertrophy associated with *NF1*. The authors posited that adding *NF1* to diagnostic panels could allow early diagnosis of patients with *NF1*. In 2012, Alkindy et al. [18] assessed a 71-year-old father and his 29-year-old son who had symptoms of echocardiographically diagnosed HCM manifesting as cardiovascular-related complications of *NF1*. Echocardiographic findings of a ratio of the free wall of the septum to the left ventricular posterior wall of greater than 1.5 (indicative of HCM) are seen in at least 4% of patients with *NF1*. Cardiac hypertrophy is the most common risk factor in mortality and cardiovascular complications in these patients. Congenital heart diseases and cardiomyopathy are also common concomitants of several growth and development syndromes that affect RASopathies. In these syndromes, increased signaling through Erk1/2 and mTOR complex 1 causes

cardiomyopathy. Neurofibromin regulates Ras signaling, and Ras activation causes progressive cardiac hypertrophy in adult mice. Our study patient was also suffering from cardiomyopathy, ACM. All such patients show *NF1* pigmentary changes with very few *NF1*-related complications milder in terms of clinical phenotypes. Our patient had more than 6 light brown spots on the skin of his legs and upper body. In 2016, Jurko et al. [9] described an 18-year-old boy suffering from *NF1* and HCM with forward systolic movements of the anterior leaflet of the mitral valve. The authors concluded that one of the common causes of severe arrhythmias and sudden deaths was HCM and that cardiac involvement caused significant problems in patients with NF, although relevant information was scarce. Patients with NF can develop progressive cardiomyopathy, hence the need for follow-ups in cardiology clinics to swiftly identify any change in the electrocardiogram or the chest X-ray. Additionally, the follow-up of HCM is necessary as it may require surgery [9]. This study holds significance in several aspects, clinical Correlation: The study found that the patient exhibited signs consistent with ACM, which is a significant complication associated with *NF1*. This correlation between the genetic variant and clinical presentation highlights the importance of genetic testing in diagnosing and managing *NF1* patients, as it can help predict potential complications and guide appropriate medical interventions. Family screening and counseling: by identifying the genetic variant in the affected individual, the study demonstrates the utility of genetic testing in identifying asymptomatic family members who may be at risk of developing *NF1*-related complications. This has implications for genetic counseling and family planning, as it allows for informed decision-making and proactive management of the condition within the family.

Conclusions

The proband in the current study had *NF1* and ACM. This investigation is the first to report ACM in a patient with *NF1*. Although there is no cure for *NF1*, genetic tests, such as WES, can identify asymptomatic family members at risk. In addition, cardiac evaluation could

assist in helping these patients before cardiac problems develop.

Accession Number.

The accession number of the variant in ClinVar is as follows:

NM_001042492.3 (NF1): c.3277G>A (p.Val1093Met): VCV000457638.5.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12872-024-03878-z>.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Acknowledgements

The authors wish to acknowledge the kindly contribution of this family. This research was provided by Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Tehran, Iran and Department of Biology, Science and research Branch, Islamic Azad University, Tehran, Iran.

Author contributions

MP, and SK drafted the work. SK performed WES analysis. MP contributed to the wet-lab. MM and GH surveyed the patients clinically. All the authors read and approved the final manuscript.

Funding

The authors received no specific funding for this research.

Data availability

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran (IR.RHC.REC.1402.017). Written informed consent was obtained from the participant family.

Competing interests

The authors declare no competing interests.

Consent for publication

Informed consent for publication was obtained from all the study participants.

Received: 25 December 2023 / Accepted: 5 April 2024

Published online: 23 April 2024

References

- McClatchey AI. Neurofibromatosis. *Annu Rev Pathol Mech Dis*. 2007;2:191–216.
- Gerber P, Antal A, Neumann N, Homey B, Matuschek C, Peiper M, et al. Neurofibromatosis. *Eur J Med Res*. 2009;14(3):102–5.
- Korf BR. Neurofibromatosis. *Handbook of clinical neurology*. 2013;11:333–40.
- Gutmann DH, Ferner RE, Listernick RH, Korf BR, Wolters PL, Johnson KJ. Neurofibromatosis type 1. *Nat Reviews Disease Primers*. 2017;3(1):1–17.
- Friedman J, Arbiser J, Epstein JA, Gutmann DH, Huot SJ, Lin AE, et al. Cardiovascular disease in neurofibromatosis 1: report of the NF1 Cardiovascular Task Force. *Genet Sci*. 2002;4(3):105–11.
- İncecik F, Hergüner ÖM, Alinç Erdem S, Altunbaşak Ş. Neurofibromatosis type 1 and cardiac manifestations. *Türk Kardiyoloji Dernegi Arsivi: Türk Kardiyoloji Derneğinin Yayın Organidir*. 2015;43(8):714–6.
- Miller DT, Freedenberg D, Schorry E, Ullrich NJ, Viskochil D, Korf BR et al. Health supervision for children with neurofibromatosis type 1. *Pediatrics*. 2019;143(5).
- Ibarrola M, Pérez-Riera AR, González MD. Left ventricular noncompaction and orthodromic atrioventricular tachycardia observed in a patient with neurofibromatosis type 1. *Oxf Med Case Rep*. 2019;2019(3):omz021.
- Jurko A Jr, Minarik M, Minarikova E, Nagi AS. Neurofibromatosis associated with hypertrophic cardiomyopathy. *Saudi Med J*. 2010;31(8):935–6.
- Nguyen R, Mir T, Kluwe L, Jett K, Kentsch M, Mueller G, et al. Cardiac characterization of 16 patients with large NF1 gene deletions. *Clin Genet*. 2013;84(4):344–9.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. *Bioinformatics*. 2009;25(16):2078–9.
- McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The genome analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297–303.
- Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res*. 2010;38(16):e164–e.
- 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 Sci*. 2015;17(5):405–23.
- Williams VC, Lucas J, Babcock MA, Gutmann DH, Korf B, Maria BL. Neurofibromatosis type 1 revisited. *Pediatrics*. 2009;123(1):124–33.
- Dunning-Davies B, Parker A. Annual review of children with neurofibromatosis type 1. *Archives Disease Childhood-Education Pract*. 2016;101(2):102–11.
- Ritter A, Cuddapah S, Degenhardt K, Kasperski S, Johnson MP, O'Connor MJ, et al. Fetal cardiomyopathy in neurofibromatosis type I: novel phenotype and review of the literature. *Am J Med Genet Part A*. 2019;179(6):1042–6.
- Alkindy A, Chuzhanova N, Kini U, Cooper DN, Upadhyaya M. Genotype-phenotype associations in neurofibromatosis type 1 (NF1): an increased risk of tumor complications in patients with NF1 splice-site mutations? *Hum Genomics*. 2012;6:1–7.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.