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CASE REPORT

Dilated cardiomyopathy associated with NRAP gene: a case series

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ABSTRACT

Background: The genetic basis of dilated cardiomyopathy (DCM) is highly diverse, with over 100 known genes and several possibilities described. Nebulin-related-anchoring protein (NRAP) is an action-binding cytoskeletal protein that has a role in the myofibrillar assembly in the embryonic heart. It is primarily generated in striated and cardiac muscles.

Case Presentation: We described three cases of DCM that were related to *NRAP* gene mutations [NM_001261463.1: c.3568G > T; p. (Glu1190*)].

Conclusion: Our data imply that biallelic nonsense mutations in the NRAP might be a genetic risk factor with limited penetrance and induce DCM at various ages.

Keywords: NRAP gene, heart failure, cardiomyopathies, DCM, genetics.

Introduction

Dilated cardiomyopathy (DCM) is differentiated in the absence of other etiological causes by left ventricular hypertrophy and systolic dysfunction (1). Even within the same family, the presentation of DCM can range from asymptomatic to heart failure in advanced stages and sudden cardiac death. The estimated frequency of DCM in the general population is between 1:500 and 1:3,000 (2).

Despite the fact that the genetic basis of DCM is still poorly understood, multiple genes have been associated with it (3). These genes code for cytoskeletal, sarcomere, nuclear envelope, ion channel, and intercellular junction proteins (4). Diverse genes have been identified as dominant loci for the condition, which appears to be hereditary in over 20% of patients (5,6). Several of these genes code for cytoskeletal proteins, indicating that inadequate force transmission is among the major causes of DCM (7). For example, a mutation in the MYH7 gene, which encodes myosin heavy chain 7, a structural protein in the heart, has been associated with DCM. Similarly, a mutation in the TNNT2 gene, which encodes troponin T, a protein involved in muscle contraction, has been linked to DCM. Other genetic risk factors for DCM include mutation in the LMNA, TNNI3, and TTN genes. Nebulin-related anchoring protein (NRAP), which is involved in the cycle of sarcomeric contraction, is one of the most recent genetic findings linked to DCM.

Heart and striated muscle-expressed NRAP is the second-largest actin-binding cytoskeletal protein in the nebulin family (8). NRAP is expressed in myofibrillogenesis by myofibril precursors. NRAP participates in myofibrillar assembly during cardiomyocyte development in the fetal heart and connects terminal actin filaments to membrane complexes in the adult heart (9,10). Experimentally, NRAP expression was upregulated in both DCM animal models and DCM patients (11).

In this case series, three confirmed cases of DCM associated with *NRAP* mutations are presented.

Case Presentation

Case 1

A 5-year-old female presented to the emergency department with persistent vomiting, epigastric discomfort, and easy fatigability for the last one and a

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half months. She was managed at a local hospital and then referred to our hospital for further management. She is the second child of a consanguineous marriage. Her family history is unremarkable. On examination, an unwell child weighed 17 kg (50th-75th centile), height of 105 cm (25th-50th centile), and had a head circumference of 50 cm (50th centile). An abdominal examination revealed hepatomegaly. On cardiovascular examination, normal heart sounds and a pan-systolic murmur with a displaced apex beat were discovered. The rest of the systemic examination was unremarkable. Her initial blood investigations were within the normal limit. Electrocardiography showed normal sinus rhythm, right axis deviation, and left ventricular hypertrophy with inverted T waves in lateral leads. Echocardiography showed severe left ventricular dilation, moderate mitral regurgitation, and an ejection fraction of 15%. She was treated as a case of DCM and started on milrinone at 0.5 µg/kg/hour, but her condition deteriorated every day. During the hospital stay, she became unwell and was shifted to the pediatric intensive care unit (ICU), where she was intubated. She was getting full inotropic support, but she later had problems with several organs and died within a month of being admitted. A homozygous autosomal recessive likely pathogenic variant in the *NRAP* gene [NM_001261463.1: c.3568G>T; p. (Glu1190*)] results in a premature stop codon (Figure 1A).

Case 2

An 11-month-old male was referred from a peripheral hospital for pediatric ICU care with a history of shortness of breath and respiratory distress. He has an uneventful neonatal history and is developmentally normal. His parents were first cousins. He has a cousin who died at the age of three from cardiac problems. He was active and alert on examination, with a glasgow coma scale (GCS) of 15/15 and no dysmorphic features. weight is 9 kg (10th-25th centile) and his height is 74.5 cm (10th centile). Head circumference: 46 (25th centile). Chest examination revealed crepitation with normal vesicular breath. An abdominal exam shows hepatomegaly. A cardiovascular examination showed a pansystolic murmur. The rest of the systemic examination was unremarkable. Echocardiography showed a severely dilated left ventricle and atrium. He has severely depressed left ventricular systolic function, with an ejection fraction (EF) estimated at 15%-17%. Severe mitral regurgitation, moderate aortic insufficiency, normal sinus rhythm, right axis deviation, left ventricular hypertrophy, ST-segment alterations, T-wave inversion, and large Q waves in the lateral precordial leads were found in the ECG lab. Inflammatory markers and metabolic profiles were unremarkable. He was admitted to the pediatrics intensive care unit (PICU) and started on heart failure treatment. He was discharged home on LASIX PO 9 mg TID, CAPTOPRIL 3.25 mg PO BID, ASPIRIN 45 mg PO OD, and DIGOXIN 50 MCG PO BID. Propranolol 4.5 PO BID. On follow-up later, we received information that his condition deteriorated at home, which resulted in his sudden death. This whole exome sequence came back showing a homozygous autosomal recessive likely pathogenic variant was identified in the *NRAP* gene (NM_001261463.1: c.3568G>T; p.(Glu1190*) which creates a premature stop codon. Thus, the obtained result is consistent with a genetic diagnosis of NRAP-related cardiomyopathy (Figure 1B).

Case 3

A 4-year-old boy with a known case of DCM and sickle cell trait presented to the emergency department with generalized body swelling, cough, and decreased urine output. His parents were first cousins. His father died of a cardiac problem. He has a normal developmental history. He had previously been admitted to the PICU for the same complaint. On examination the following observations were made: he was afebrile, had a pulse of 130 bpm, blood pressure of 83/41 mmHg, had cold extremities, had capillary refills >2 seconds; GCS 15/15, no dysmorphic features, 11 kg (3rd centile), and height 92 cm (10th centile). Examination revealed abdominal distention and tender hepatomegaly. Chest examination showed bilateral crepitation with bronchovesicular breathing. He has a pansystolic murmur with a displaced apex beat on the background of normal S1 and S2. Electrocardiography showed normal sinus rhythm, right axis deviation, and left ventricular hypertrophy, with inverted T waves in the lateral lead. Echocardiography showed severe left ventricular depression with an 18% ejection fraction. He was admitted to the PICU and started on heart failure treatment. The full genetic workup-initiated report revealed an *NRAP* gene mutation and patient transfer to a high-level cardiac transplant center (Figure 1C).

Discussion

NRAP is essential for the assembly of thin filaments, actin cytoskeleton organization, and myofibril assembly in cardiomyocytes. NRAP attaches to the membrane, facilitating cardiac muscle contraction and relaxation via a complex of -integrins, talin, and vinculin. It is anticipated that these super repeats will give directionality to actin filaments (12).

We report the discovery of a novel variant in the *NRAP* gene in three patients from unrelated families with confirmed DCM. In all three cases, a homozygous likely pathogenic variant was recognized within the *NRAP* gene NM_001261463.1: c.3568G>T variant p. (Glu1190*). The OMIM phenotype has never been reported to have been linked to the pathogenic variants in the N-RAP gene. Again, based on a review of the clinical database, we found 172 variants in the *NRAP* gene. Of them, 22 reported variants in *NRAP* were classified as pathogenic and 2 as likely pathogenic. The rest are either benign or variants of uncertain significance. Nevertheless, numerous studies show that many patients with cardiomyopathy have the *NRAP* gene consisting of a loss-of-function variant. The three case reports align with the findings of various reports that associate homozygous mutations within the *NRAP* gene with cardiomyopathy (13,14).

However, only two homozygous LoF variations have been documented in the literature. Ahmed et al. (15) described a 13-month-old female with an *NRAP*-associated mutation, and the second instance was reported in a previously healthy 26-year-old woman (12). In our cases, all were younger than 6 years old, had a positive consanguinity

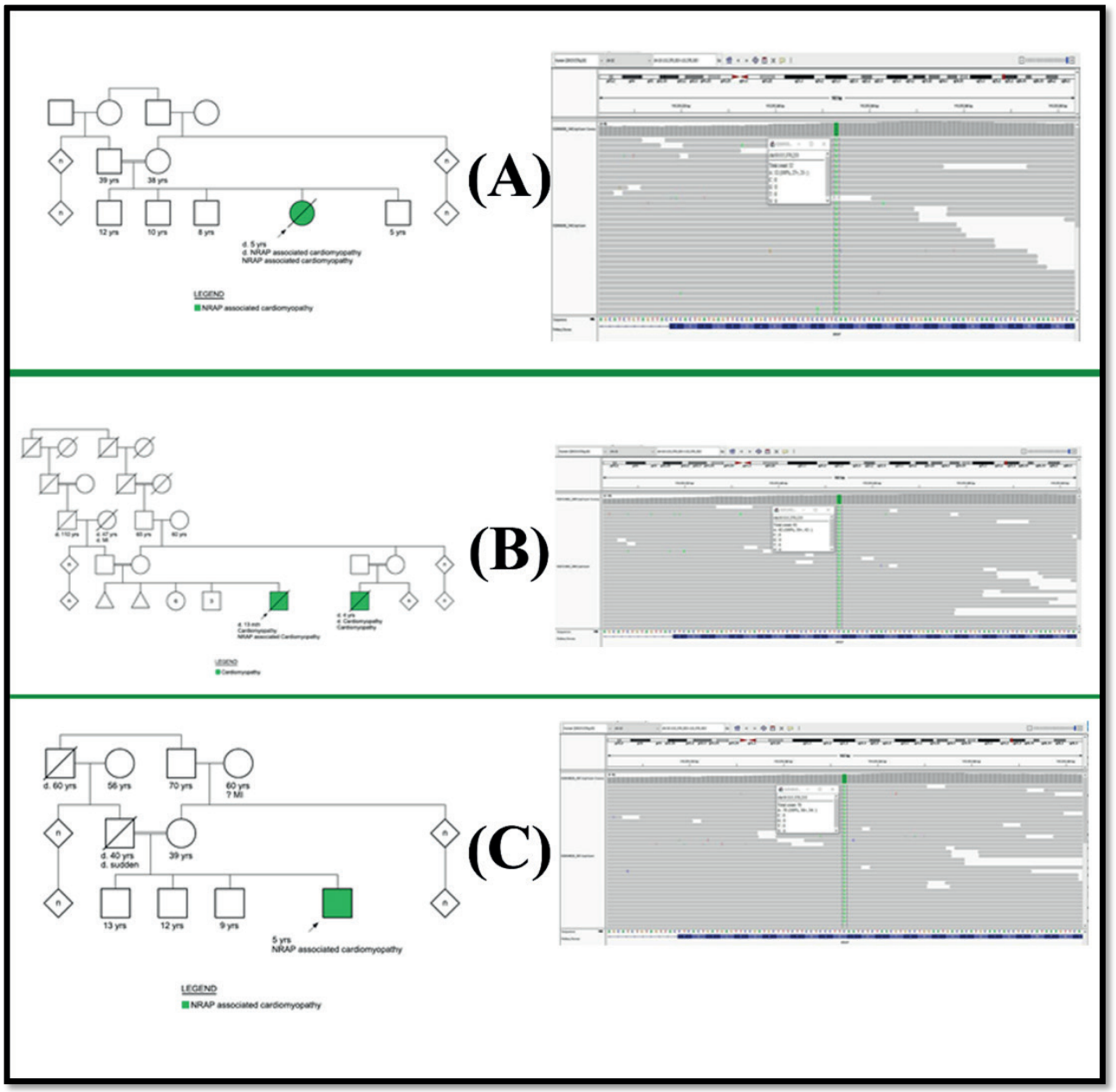

Figure 1. *(A) A family pedigree and a chromatograph of the mutations of Case 1 with NRAP-associated cardiomyopathy. (B) A family pedigree and a chromatograph of the mutations of Case 2 with NRAP-associated cardiomyopathy. (C) A family pedigree and a chromatograph of the mutations of Case 3 with NRAP-associated cardiomyopathy.*

test, and were reported to the emergency department with signs of heart failure. Ahmed et al. (15) documented a consanguineous pedigree in which the index patient was a 13-month-old girl diagnosed with DCM who presented with heart failure, easy fatigability, weakness, irritability, and shortness of breath. Her healthy 33-year-old father was found to be homozygous for the same frameshift variation detected in the proband, while her mother was heterozygous. This finding may explain the different ages of presentation. Unfortunately, we were not able to perform a genetic test for one of the parents of the affected child, who passed away suddenly from cardiac arrest.

One of our cases is an 11-month-old boy who supports that NRAP has a crucial role in enabling myofibrillogenesis within early childhood. The *NRAP* gene expressed in cardiac and striated muscles is known to be the second largest member of the actin-binding cytoskeletal proteins. Zhang et al. (16) indicated that the development of cardiomyocytes within the fetal heart engages N-RAP in the myofibrillar collection. It also connects terminal actin filaments towards an adult heart's membrane complexes to force transmission between the sarcomere and extracellular matrix. Homozygosity for rs530462185 was indicated even in the asymptomatic boy. This shows that the mutation of N-RAP in people can be tolerated due to the limited penetrance and must need other factors to cause an illness. The idea that the N-RAP is inadequate to cause disease is also supported by a study conducted by Bielecka-Dabrowa et al. (17) and D'Avila et al. (18); nevertheless, the deaths of the two patients in the case study indicate that a homozygous

gene in N-RAP was inherited genetically by the patients to cause cardiomyopathy (14). This was identified through the double-stranded DNA detection method, which was conducted against the human coding exome. Also, clinical data and family history information are essential as they help to assess the noted variants by referring to their causality and pathogenicity. In conclusion, the report presents three different cases of patients diagnosed with cardiomyopathy.

Conclusion

This case series confirms the clinical and natural history of this disease and its fatal prognosis. We proposed international registries to study *NRAP* gene-related DCM and collect larger cohorts that would help us characterize the disease and make genotype-phenotype correlations.

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The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Consent for publication
Due permission was obtained from the parents of the patient to publish the cases.

Ethical approval
Ethical approval is not required at our institution to publish an anonymous case report.

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