Review Article

Exploring the Role of *ALPK3* Variants in Hypertrophic and Dilated Cardiomyopathies

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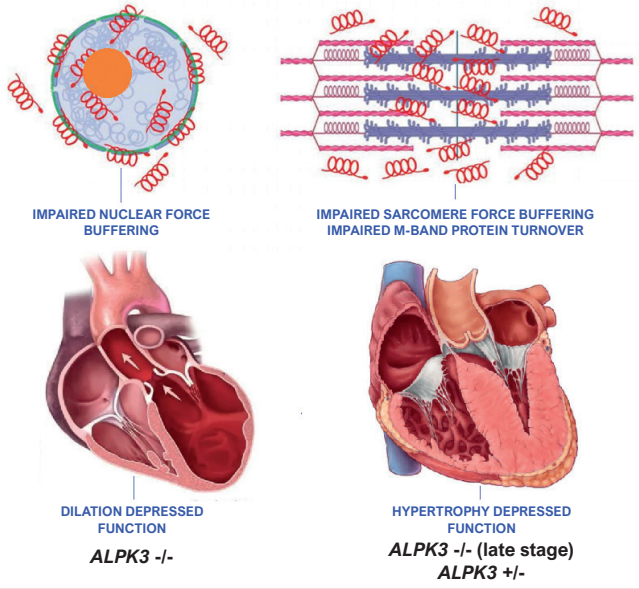
Central Illustration: Exploring the Role of *ALPK3* Variants in Hypertrophic and Dilated Cardiomyopathies



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Loss-of-function (LOF) patogenic variants in ALPK3 result in the improper localization and accumulation of myomesin proteins, impairing their role in force buffering at both the nuclear envelope and sarcomere. This disruption causes dysfunction in cardiomyocytes, leading to ventricular dilation and rapid functional decline (end-stage heart failure). In neonatal survivors and adults with heterozygous ALPK3 variants, hypertrophy may arise from dysregulation of other M-band proteins, such as decreased MuRF1 and increased CAPN3 levels, which are critical for sarcomeric protein turnover. (Inspiration and adaptation of artwork – Agarwal R. et al.[1]). ALPK3 (alpha-kinase 3); CAPN3 (calpain 3); MuRF1 (muscle RING-finger protein 1); WT (wild type); red spirals (myomesin proteins); structures of the actin-myosin system in purple and pink (dysregulation of other M-band proteins).

Keywords

Hypertrophic Cardiomyopathy; Dilated Cardiomyopathy; Sarcomeres; Population Biological Variation

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Abstract

Alpha-protein kinase 3 (*ALPK3*) variants, particularly truncating mutations (*ALPK3*tv), have been associated with both hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), contributing to phenotypic variability and severe clinical outcomes. HCM is characterized by left ventricular hypertrophy (LVH) not attributable to other causes and is a common genetic disorder, often linked to pathogenic variants in sarcomeric genes. However, clinical genetic testing fails to identify a causative mutation in approximately 40% of the cases, suggesting the involvement of non-sarcomeric genes such as *ALPK3*.

Recent studies have revealed that biallelic *ALPK3*tv is associated with severe early-onset cardiomyopathy. Patients may exhibit a clinical progression from DCM in infancy to HCM, with impaired systolic function in later life. The HCM phenotype in these individuals is often characterized by extensive myocardial fibrosis, apical or concentric patterns of left ventricular hypertrophy, and a low prevalence of left ventricular outflow tract obstruction. *ALPK3*tv carriers are also at risk for progressive myocardial dysfunction, heart failure, and, in some cases, sudden cardiac death.

The molecular mechanisms underlying *ALPK3*-related cardiomyopathy involve the disruption of key structural and regulatory proteins, such as myomesins, which are essential for sarcomere integrity and cardiomyocyte force buffering. The discovery of *ALPK3* variants in underrepresented populations further highlights the importance of genetic research in diverse cohorts. Understanding the clinical and molecular consequences of *ALPK3* variants could lead to more precise diagnostic and therapeutic approaches for cardiomyopathies associated with this gene.

ALPK3 and Cardiomyopathies

Alpha-protein kinase 3 (*ALPK3*) gene, located on chromosome region 15q25.2, has recently been linked to various cardiomyopathies, depending on the specific variants present. Hypertrophic cardiomyopathy (HCM) is defined as left ventricular hypertrophy (LVH) that other conditions cannot explain. It is a genetic disease affecting approximately 1 in 500 individuals, as well as one of the leading causes of sudden cardiac death (SCD).[2] HCM is primarily inherited as an autosomal dominant condition resulting from pathogenic variants in cardiac sarcomere genes. Despite the availability of clinical genetic testing, pathogenic or likely pathogenic variants are identified in only approximately 60% of HCM cases, highlighting the potential for further genetic discoveries. Biallelic truncating variants in *ALPK3* (*ALPK3*tv) were initially reported in 2000, with their role in non-sarcomeric HCM being definitively established in 2016. These variants are particularly important for diagnosing non-sarcomeric forms of HCM, which are associated with more severe clinical outcomes. Additionally, some cases exhibit phenotypic progression from dilated cardiomyopathy (DCM) during infancy to HCM, with systolic dysfunction later in life. Variants in *ALPK3* are associated with significant phenotypic variability, with HCM often presenting as apical or concentric left ventricular hypertrophy (LVH), minimal left ventricular outflow tract obstruction, and extensive fibrosis detectable through cardiac magnetic resonance imaging.[2,3]

ALPK3: Structure and Function

Molecular Structure and Function

ALPK3 is a protein-coding gene that has not been extensively studied. It is thought to phosphorylate key cardiac transcription factors, such as HEY2, and influence cardiomyocyte differentiation. Evidence from recent studies indicates that *ALPK3* is involved in the structural organization of intercalated discs and sarcomeres.[3] Mouse models lacking *ALPK3* function show ventricular hypertrophy and impaired contractility, while cardiomyocytes derived from stem cells with homozygous *ALPK3* variants exhibit altered calcium handling.[1,3]

Gene and Protein Composition

The human *ALPK3* gene consists of 14 exons, encoding a 1907 amino acid protein with an alpha-type protein kinase domain and two immunoglobulin-like domains. Though its precise role remains unclear, *ALPK3* may act as a regulator of cardiac transcription factors, such as HEY2. As a non-sarcomeric gene, it interacts with myomesins—proteins expressed in both the nuclear membrane during early development and later in the sarcomere. Alterations in this gene can disrupt the structural organization of myomesins, leading to impaired force buffering between cardiomyocytes and resulting in metabolic dysregulation and reduced cellular turnover (Figure 1). [2,4,5]

ALPK3 Variants and Cardiomyopathy

Depending on the variant, *ALPK3*-related cardiomyopathy can manifest as either DCM, resulting from compromised force buffering by myomesins, or HCM, caused by sarcomeric dysfunction. Biallelic truncating mutations lead to severe, early-onset cardiomyopathy. These variants impair contractility and cause ventricular dilation due to mislocalization and dysregulation of myomesin, critical components for maintaining force in cardiomyocytes. Additionally, hypertrophy may result from dysregulation of M-band proteins, which are essential for sarcomere protein turnover. Therapeutic approaches that restore cardiomyocyte force buffering and sarcomere turnover could improve outcomes in patients with *ALPK3*-related cardiomyopathy.[6,7]

ALPK3 Truncating Variants

Clinical findings

A case report has described a family with HCM caused by a heterozygous *ALPK3* variant, and truncating variants of *ALPK3* associated with autosomal dominant inheritance were found to be enriched in a mixed cohort of patients with both HCM and DCM. In a study by Lopes et al. (2021), whole-exome sequencing of a cohort of patients with HCM revealed that *ALPK3* truncating variants cause severe clinical phenotypes, including extensive myocardial fibrosis and progression to heart failure in adulthood, as exemplified in the cardiac MRI shown in Figure 2, where the extensive fibrosis resulted in sudden cardiac death in the patient in question. Histopathological analysis confirmed significant fibrosis and cardiomyocyte vacuolation, with minimal myocyte disarray. [8,9]

Incomplete penetrance and variable presentation

Clinical descriptions of heterozygous carriers of *ALPK3* truncating variants vary, with only 5 of 37 carriers meeting the diagnostic criteria for HCM. Incomplete age-related penetrance, commonly seen in autosomal dominant HCM

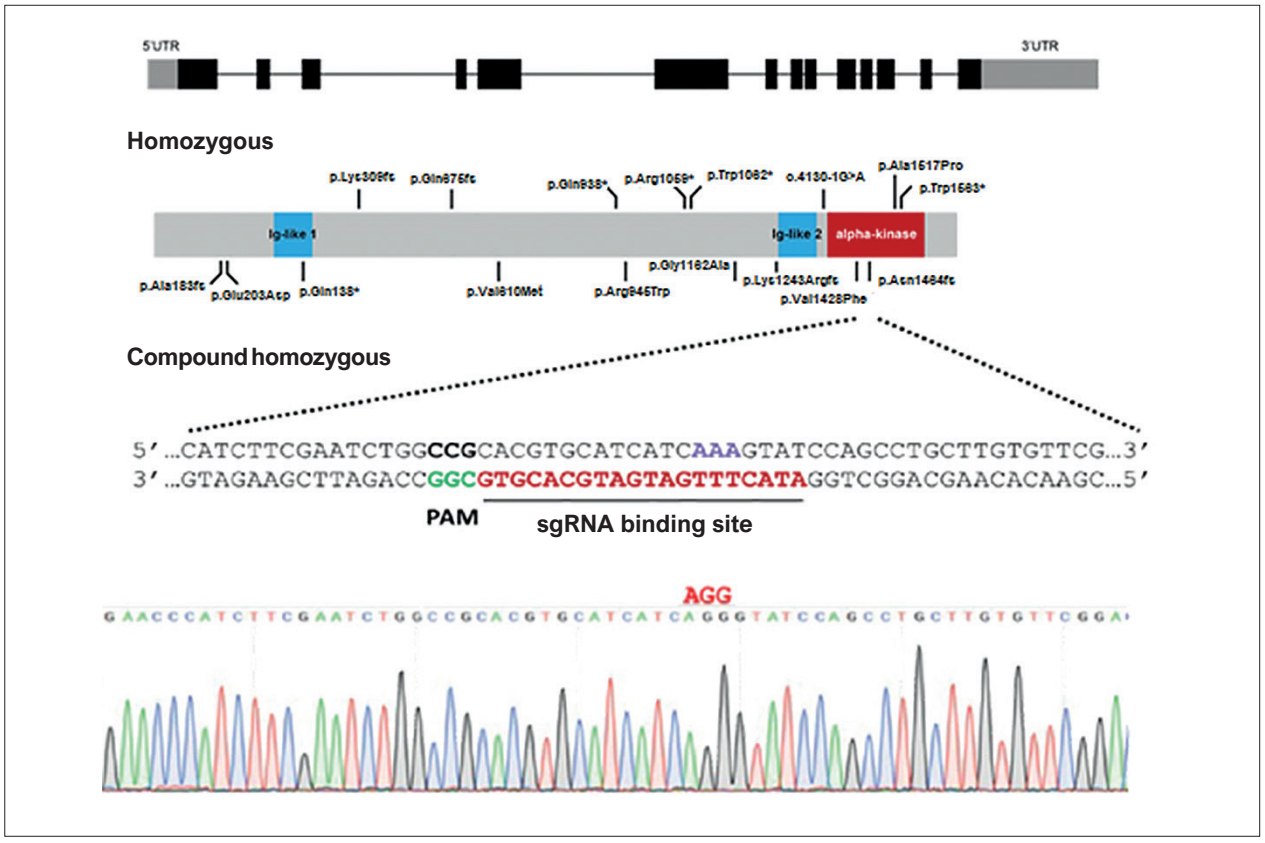

Figure 1 – *Schematic representation of the ALPK3 gene structure (top), as well as the protein and the location of disease-associated variants (Figure adapted from the canonical transcript NM_020778.5).*

genes, may explain this variability. Further studies are needed to clarify the association between *ALPK3* variants and skeletal muscle involvement, as reported cases show variability in findings, including myopathy and altered creatine kinase levels.[10]

Ethnic and population considerations

Lopes et al. (2021) observed that the frequency of *ALPK3* truncating variants was significantly higher in patients of South Asian ancestry with HCM (6.8%) compared to Europeans (1.6%), despite similar population frequencies in the gnomAD database. This suggests a higher penetrance of these variants in South Asian populations.[8-10]

ALPK3 in homozygosity

Biallelic variants and phenotypic progression

ALPK3-related cardiomyopathy is characterized by phenotypic variability. Initially identified in five children with severe early-onset cardiomyopathy, recent studies have expanded the cohort to 19 patients, many of whom presented with DCM that later progressed to HCM. These patients often exhibit extracardiac manifestations, such as scoliosis, facial dysmorphism, and cleft palate, although these are not consistent enough to define a recognizable syndrome. The clinical course is frequently severe, with several patients requiring heart transplantation or succumbing to the disease.[12-14]

Missense variants and protein dysfunction

Biallelic missense variants causing pediatric-onset cardiomyopathy have also been identified. These variants likely lead to conformational changes in *ALPK3*, affecting protein folding, flexibility, or interactions with other proteins and DNA. However, the lack of a 3D structure for *ALPK3* makes it difficult to establish accurate *in silico* predictions on the consequences of missense variants. Herkert et al. (2020) reported that approximately half of pediatric patients with *ALPK3* variants experienced a progression from DCM to ventricular hypertrophy, with some cases showing a mixed cardiomyopathy phenotype that evolved into concentric hypertrophy of both ventricles (Central Illustration).[1,13-16]

Histopathology

Histological features and arrhythmogenic characteristics

Histopathologically, biallelic *ALPK3* cardiomyopathy shares some features with classical HCM, such as focal cardiomyocyte hypertrophy, interstitial fibrosis, and myocardial fiber disorganization. Rhythm and conduction abnormalities

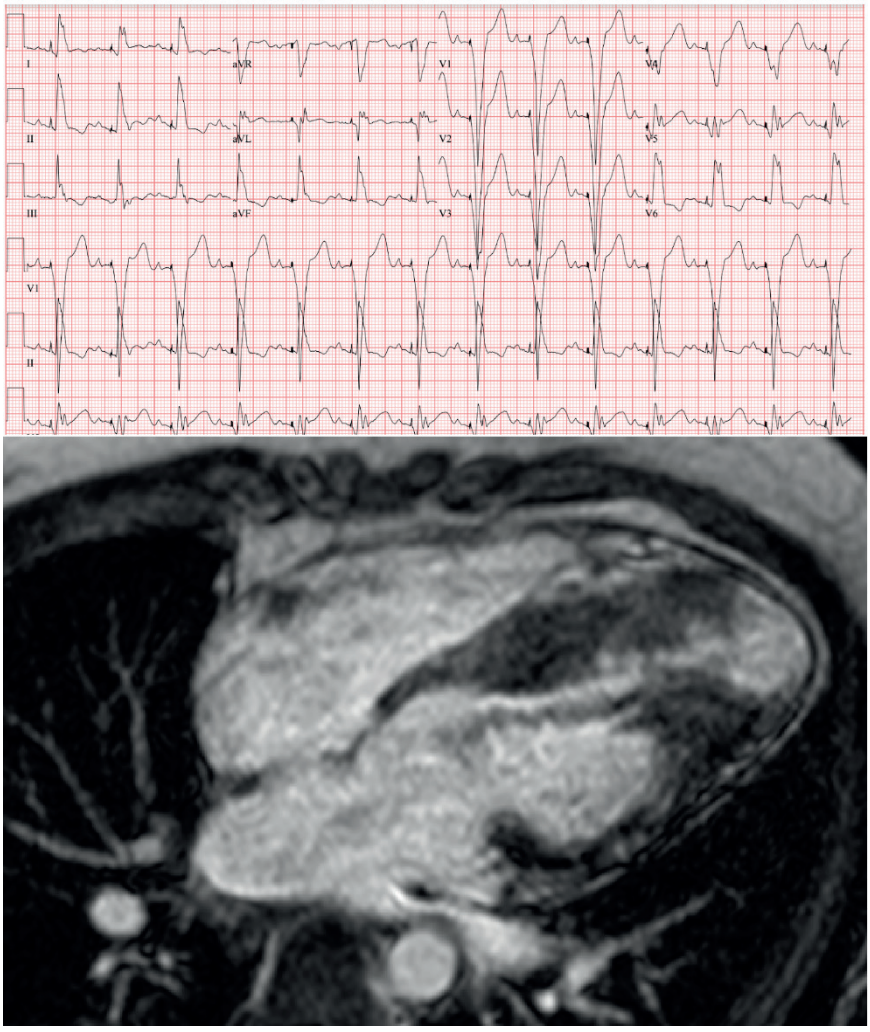

Figure 2 – *Electrocardiogram shows a pacemaker rhythm. Cardiac magnetic resonance imaging reveals mid-apical left ventricular hypertrophy with extensive fibrosis. (The images used in this work were generously provided by the author's personal library. These images are part of a private collection curated by the author and have been used with explicit permission for this purpose.)*

resembling arrhythmogenic cardiomyopathy (ACM) are also common. Reduced plakoglobin signaling at intercalated discs, similar to ACM, suggests a shift from junctional to intracellular and nuclear compartments, inhibiting canonical Wnt/beta-catenin signaling. This overlap may explain arrhythmogenic features in *ALPK3* cardiomyopathy.[10-12]

Implications

Prognosis and clinical management

Both carriers of *ALPK3* truncating variants and patients without identifiable genetic variants - who develop heart failure due to other causes - can progress to end-stage heart failure at comparable rates. However, the presence of *ALPK3* truncating variants seems to uniquely predispose individuals to a more pronounced and progressive myocardial dysfunction. This suggests that, while the ultimate outcome of heart failure may be similar, the underlying genetic defect in *ALPK3* may accelerate the deterioration of myocardial function or amplify pathological processes, leading to a more aggressive disease course in affected individuals.

The incidence of end-stage heart failure in carriers of the *ALPK3* variant is similar to that in patients without detectable pathogenic variants but with a higher prevalence of myocardial scarring and heart transplant referrals. These findings indicate that *ALPK3* truncating variants significantly elevate the risk of progressive myocardial dysfunction.[4,8,9]

Genetic counseling and screening

Genetic testing extends beyond the index patient to benefit family members through early diagnosis and intervention. Screening of first-degree relatives of patients with HCM or

DCM, particularly those with *ALPK3* variants, is essential for early detection and management. Finally, the growing evidence of this gene being associated not only with autosomal recessive but also autosomal dominant inheritance brings major implications regarding the risk of recurrence and, therefore, family planning of such patients.[4,8]

Take-home messages

- ***ALPK3* Variants and Cardiomyopathy.** Mutations in *ALPK3*, a gene highly expressed in muscle tissues, are associated with both neonatal and adult-onset forms of cardiomyopathy. These mutations can lead to ventricular dilation or hypertrophy. Despite containing an alpha-kinase domain, *ALPK3* functions as a pseudokinase and lacks catalytic activity, underscoring its unique role in disease development.
- **Protein Localization and Function.** *ALPK3* localizes to both the nuclear envelope and the M-band of the sarcomere in cardiomyocytes. It plays a crucial regulatory role in the expression and positioning of myomesins (MYOM1 and MYOM2), proteins essential for maintaining the structural integrity and turnover of sarcomeric components. Disruptions in *ALPK3* function impact the structural organization of these proteins, contributing to cardiomyopathy.
- **Impact on Contractility and Dilation.** *ALPK3*-related cardiomyopathy can impair heart muscle contractility and cause ventricular dilation due to the mislocalization and dysregulation of myomesin proteins, which are critical for force buffering in cardiomyocytes. This impaired force buffering compromises the heart's mechanical stability and function.
- **Hypertrophy and Disease Progression.** In addition to causing dilation, *ALPK3* mutations can promote cardiac hypertrophy by disrupting the regulation of key M-band proteins involved in sarcomere protein turnover. This leads to excessive sarcomeric buildup, which is a hallmark of hypertrophic cardiomyopathy.
- **Therapeutic Potential.** Therapies aimed at restoring the normal force-buffering functions of cardiomyocytes and improving sarcomere protein turnover may offer promising avenues for treating patients with *ALPK3*-related cardiomyopathy. Such strategies could potentially mitigate disease progression and improve patient outcomes.

Conclusion

The study of *ALPK3* highlights the critical need to expand genetic research beyond European populations to include underrepresented groups. The identification of rare biallelic truncating variants in *ALPK3*, particularly in regions with high consanguinity, underscores the importance of broadening our understanding of the genetic basis of cardiomyopathies. These discoveries open new avenues for identifying autosomal dominant HCM in diverse populations, which may improve diagnosis, management, and outcomes for affected individuals.

Author Contributions

Conception and design of the research and Writing of the manuscript: Andrade FA, Stephan BO; Acquisition of data: Andrade FA; Analysis and interpretation of the data: Stephan BO, Furquim SR, Krieger JE; Critical revision of the manuscript for content: Andrade FA, Stephan BO, Furquim SR, Linnenkamp BDW, Pires LVL, Val VP, Buriti NA, Lipari LFVP, Correia VM, Krieger JE.

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Study association

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Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

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