EDITORIAL FOCUS

The PRKAG2 gene and hypertrophic cardiomyopathy: an energetically imbalanced relationship

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HYPERTROPHIC CARDIOMYOPATHY (HCM) is the most common inherited cardiac disease, characterized by left ventricle thickening, myocardial disarray, and sudden death (14). Its clinical spectrum spans from an asymptomatic state to outflow tract obstruction, diastolic dysfunction, tachyarrhythmias, and progressive heart failure.

Pediatric forms of the disease have been associated with a lower risk of sudden death in the 2- to 7-yr-old age group and with a higher risk between 8 and 16 yr of age (18). On the other hand, HCM is mostly an adulthood disease and is considered the primary cause of sudden death among athletes (15).

Since pathogenic variants have been detected in most sarcomeric proteins, 70% of the identified mutations actually involve the myosin-binding protein-C3 and myosin heavy chain 7 (MYH7) genes (5, 10, 11, 17). HCM is frequently described as a disease of the sarcomere, the molecular machinery responsible for cardiac contraction. However, mutations have also been detected in other genes, leading to the presence of other distinctive phenotypes associated with HCM, such as conduction system abnormalities, mitochondrial dysfunctions, and noncardiac manifestations (22). Mutations in the protein kinase AMP-activated noncatalytic subunit-γ2 (PRKAG2) gene have been related to a variety of phenotypes, including glycogen accumulation, Wolff-Parkinson-White syndrome, conduction system disease, and HCM (2, 24), which in this case is associated with an atypical distribution of hypertrophy and a higher rate of heart failure and arrhythmic complications (4, 20). The PRKAG2 gene encodes for the γ2-regulatory subunit of AMP-activated protein kinase (AMPK), an enzyme that increases the amount of ATP available for metabolic activity (23). Taken together, these observations underline the complexity of the genetic causes and phenotypic variability associated with HCM.

The fact that the same disease can be determined by mutations occurring in genes encoding for proteins belonging to distinct subcellular systems indicates that in HMC, there is no “unifying” abnormality of the cardiac contractility. For example, some mutant proteins have been found to correlate with an increase of Ca2+ sensitivity and contractility (e.g., troponin T or α-tropomyosin), whereas others have the opposite effect (16). This suggests that the alteration of contractility alone doesn’t directly determine HCM and that other myocardial abnormalities must be involved. Interestingly, sarcomeric mutations in HCM have been associated with an inefficient use of ATP (6), which might arise from the inability of the cells to maintain normal ATP levels; similarly, alterations in PRKAG2 also result in a compromised energy supply (12). Hence, ATP deficiency has been identified as a potential common event in HCM arising from mutations in different subsets of genes.

In a recent article in the American Journal of Physiology-Heart and Circulatory Physiology, Xu et al. (25) reported a description of the p.K475E de novo heterozygous variant in the PRKAG2 gene in a child affected with HCM, resulting in a change of a positively charged lysine with a negatively charged glutamate. Interestingly, this is the first report of a mutation occurring in the cystathionine β-synthase 3 (CBS3) domain of the AMPK γ2-subunit directly involved in the binding of AMP and ATP (8, 9), which might help explaining the mechanistic implications of PRKAG2 gene mutations in HCM.

The main insight from this study comes from in vitro assays performed in human embryonic kidney (HEK)-293 and H9c2 cells transformed with the mutant cDNA as well as primary fibroblasts from the patient. In HEK-293 cells, overexpression of the mutated protein led to an increase of Thr172 phosphorylation and of AMPK activity together with a reduced sensitivity to AMP in allostERIC activation and the prevention of the increase in Thr172 phosphorylation in response to phenformin, a biguanide known to enhance AMPK activity without increasing AMP/ATP (27). In contrast, in H9c2 cells and in the patient’s fibroblasts, phosphorylation was decreased for Thr172 and for p70S6 kinase and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), transcription factors involved in the mechanistic target of rapamycin (mTOR) pathway and known to regulate protein synthesis and cell growth (19). Importantly, overexpression of the mutation in H9c2 cells resulted in hypertrophy, which could be explained by the increased p70S6K and 4E-BP1 basal phosphorylation, and this effect could be reverted by rapamycin treatment. These data are in agreement with results arising from the study of a transgenic mouse overexpressing the AMPK mutation p.N488I in the heart, which showed cardiac hypertrophy and ventricular preexcitation together with mTOR complex I hyperactivation and hyperphosphorylation of p70S6K and 4E-BP1 (13). It should be noticed that the different results observed between HEK-293 and H9c2 cells regarding the phosphorylation of Thr172, increased in the former and decreased in the latter, renders the interpretation of the data less straightforward. According to the authors, such discrepancy, which definitely grants further investigations, should be ascribed to the influence of the cell environment, which might
be significantly different in HEK-293 cells compared with rat embryonic cardiomyocytes.

The emerging hypothesis for the pathogenic mechanism induced by the p.K475E mutation revolves around the disruption of electrostatic interactions with adenine nucleotides, leading to changes in the AMPK complex and reduction of basal AMPK activity. This in turn would cause the subsequent increase of p70S6K and 4E-BP1 phosphorylation, the activation of the mTOR pathway (inhibited by AMPK in normal conditions), and eventually to cardiac hypertrophy (Fig. 1). The data reported by Xu and colleagues hence provide new insights in the understanding of the pathogenic mechanism connecting the p.K475E mutation in PRKAG2 to the development of HCM. Further studies will be required to understand whether increased mTOR activity is part of the specific chain of events started by the defect in PRKAG2 and culminating with HCM or is instead a common feature of HCM pathogenesis regardless of the causative mutation.

It is not uncommon that mutations in the same gene can lead to different functional ramifications or clinical phenotypes. The p.R531Q and p.R384T pathogenic variants in AMPK determine infant congenital hypertrophic cardiomyopathy, glycogen storage, and “pseudophosphorylase kinase (PHK) deficiency” via the increase of Thr172 phosphorylation and basal activity and the reduction of AMP and ATP binding (1). Moreover, the p.G100S and p.R302G mutations have been associated with a reduction of AMPK activity in combination with glycogen metabolism dysregulation (26), whereas the p.T400N mutation caused an early increase of AMPK followed by a reduction and recovery to wild-type levels (3). In the present study, a reduction in Thr172 phosphorylation and inhibition of AMPK was associated with HCM, a finding that could provide new insights not only in the pathophysiology of HCM determined by PRKAG2 mutations but also in the genotype-phenotype correlation, which might be strictly dependent on the involved protein domain. Once again, a conclusive understanding of the mechanistic relationship between PRKAG2 mutations and HCM or other diseases (e.g., Wolff-Parkinson-White syndrome, conduction system disease, or glycogen accumulation) will require more effort.

The results reported by Xu and colleagues also have important clinical implications that could prove crucial for the future management of HCM. At present, this disease is treated with the same palliative drugs used for other inherited cardiomyopathies (e.g., arrhythmogenic cardiomyopathy and dilated cardiomyopathy), which do not halt the disease progression and do not take into account the different mechanisms associated with the distinct phenotypes (21); in the most severe cases, patients must receive the implantation of a cardioverter defibrillator to prevent sudden cardiac death. Only recently a novel promising HCM-specific drug, MYK-461, has been tested in a murine model of HCM, resulting in attenuation of hypertrophic phenotype and gene expression in mice carrying mutations in MYH7 (7). The forms of HCM determined by mutations in nonsarcomeric genes, on the other hand, have no mechanism-specific therapeutic molecules at present. In view of the results presented by Xu and colleagues, it is tempting to think that inhibition of the mTOR pathway could result in the attenuation of the pathogenic phenotype, thus opening new avenues toward the development of mutation-specific therapeutic approaches. This raises new possibilities to provide a novel class of personalized treatments that take into account the individual variability, allowing to abandon palliative drugs, still used to treat different classes of cardiomyopathies.

In conclusion, the present work describes a new mutation in the PRKAG2 gene and provides new insights into the mechanistic events leading to HCM. From here, the next goal of the field should be to confirm these results in in vitro models that more closely reproduce the functional cardiomyocyte (e.g., neonatal and/or induced pluripotent stem cell-derived cardiomyocytes) and eventually in in vivo models for HCM. This latter approach will allow us to conclusively establish the involvement of the mTOR pathway in HCM consequent to mutations in the PRKAG2 gene or in other nonsarcomeric genes.

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AUTHOR CONTRIBUTIONS
M.C. interpreted results of experiments; M.C. prepared figures; M.C. drafted manuscript; M.C. edited and revised manuscript; M.C. approved final version of manuscript.

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Fig. 1. Possible pathogenic mechanism induced by the p.K475E mutation in H9c2 cells. On the one hand, the reduction of AMP-activated protein kinase (AMPK) phosphorylation at Thr172 (P-T172) results in reduced AMPK activity and loss of mechanistic target of rapamycin (mTOR) inhibition. In parallel, the increase of the phosphorylation of the cell growth regulator p70S6 kinase (p70S6K) and the translation repressor eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) results in the suppression of the inhibition of eIF4E, enhanced cell growth, and hypertrophic cardiomyopathy (HCM) development. Cell treatment with the mTOR inhibitor rapamycin results in the reversion of cell hypertrophy.