Articles in PresS. Am J Physiol Heart Circ Physiol (June 16, 2017). doi:10.1152/ajpheart.00316.2017

PRKAG2 gene and hypertrophic cardiomyopathy: an energetically imbalanced relationship

[1] *Martina Calore, Ph.D.*

[1]*Department of Cardiology, CARIM School for Cardiovascular Diseases, Maastricht University, 6229 ER Maastricht, The Netherlands*

Correspondence should be addressed to M.C.

Manuscript information:
Body text: 1402 words (without Figure Legends and References)
27 references
1 Figure

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease, characterised by left ventricle thickening, myocardial disarrangement, 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-year-old age group and with a higher risk between 8 and 16 years of age (18). On the other hand, HCM is mostly an adulthood disease and it 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 MYBPC3 and 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 been also detected in other genes, leading to the presence other distinctive phenotypes associated with HCM, such as conduction system abnormalities, mitochondrial dysfunctions, and non-cardiac manifestations (22). Mutations in PRKAG2 gene have been related to a variety of phenotypes, including glycogen accumulation, Wolff-Parkinson-White (WPW) syndrome, conduction system disease, and HCM (2, 24), which in this case is associated with atypical distribution of hypertrophy and a higher rate of heart failure and arrhythmic complications (4, 20). The PRKAG2 gene encodes for the $\gamma 2$ regulatory subunit of adenosine monophosphate (AMP)-activated protein kinase (AMPK), an enzyme that increases the amount of adenosine triphosphate (ATP) available for metabolic activity (23). Altogether, these observations underline the complexity of the genetic causes and the 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 sub-cellular 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 calcium sensitivity and contractility (*e.g.,* troponin T or □-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 this issue of *American Journal of Physiology-Heart and Circulatory Physiology*, Xu et al. report the description of the p.K475E *de novo* heterozygous variant in the PRKAG2 gene in a child affected with HCM, resulting in the change of a positively-charged Lysine with a negatively charged Glutamate (25). Interestingly, this is the first report of a mutation occurring in the cysthationine 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 implication of PRKAG2 gene mutations in HCM.

The main insight from this study comes from *in vitro* assays performed in HEK293 and H9c2 cells transformed with the mutant cDNA, as well as primary fibroblasts from the patient. In HEK293 cells, overexpression of the mutated protein led to an increase of T172 phosphorylation and of AMPK activity, together with a reduced sensitivity to AMP in allosteric activation and the prevention of increase in T172 phosphorylation in response to phenformin, a biguanide known to enhance AMPK activity without increasing AMP/ATP (27). On the contrary, in H9c2 cells and in patient's fibroblasts phosphorylation was decreased for T172 and augmented for p70S6K and 4E-BP1, transcription factors involved in the mTOR pathway and known to regulate protein synthesis and cell growth (19). Importantly, the overexpression of the mutation in H9c2 cells resulted in hypertrophy, which could be explained by the

increased p70S6K and 4E-BP1 basal phosphorylation, and such 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, showing cardiac hypertrophy and ventricular pre-excitation, together with mTORC1 hyperactivation and hyperphosphorylation of p70S6K and 4E-BP1 (13). It should be noticed that the different results observed between HEK293 and H9c2 cells regarding the phosphorylation of T172, increased in the first 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 human embryonic kidney cells compared to 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 an increased mTOR activity is part of the specific chain of events started by the defect in PRKAG2 and culminating with HCM, or it 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 “pseudo PHK deficiency” via the increase of

T172 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), while p.T400N caused an early increase of AMPK, followed by a reduction and recovery to wild-type levels (3). In the present study, a reduction in T172 phosphorylation and inhibition of AMPK has been associated with HCM, a finding that could provide new insights not only in the pathophysiololgy 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, conduction system disease, glycogen accumulation) will require more effort.

The results reported by Xu and Colleagues also have important clinical implications, which 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 non-sarcomeric 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 mTOR pathway could result in the attenuation of the pathogenic phenotype, thus opening new venues towards 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 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 iPS-derived cardiomyocytes), and eventually in *in vivo* models for HCM. This latter approach will allow to conclusively establish the involvement of the mTOR pathway in HCM consequent to mutations in PRKAG2 gene or in other non-sarcomeric genes.

ACKNOWLEDGEMENTS

M.C. is supported by a *Marie Skłodowska-Curie* postdoctoral fellowship from the European Commission.

Legend to Figure 1: Possible pathogenic mechanism induced by the p.K475E mutation in H9c2 cells. On the one hand, the reduction of AMPK phosphorylation at T172 results in reduced AMPK activity and loss of mTOR inhibition. In parallel, the increase of the phosphorylation of the cell growth regulator p70S6K and the translation repressor 4E-BP1 results in the suppression of the inhibition of the eukaryotic translation initiation factor eIF4E, enhanced cell growth and HCM development. Cell treatment with the mTOR inhibitor rapamycin results in the reversion of cell hypertrophy.

REFERENCES

1. **Akman HO, Sampayo JN, Ross FA, Scott JW, Wilson G, Benson L, Bruno C, Shanske S, Hardie DG, Dimauro S.** Fatal infantile cardiac glycogenosis with phosphorylase kinase deficiency and a mutation in the gamma2-subunit of AMP-

activated protein kinase. *Pediatr Res* 62: 499-504, 2007.

2. **Arad M, Benson DW, Perez-Atayde AR, McKenna WJ, Sparks EA, Kanter RJ, McGarry K, Seidman JG, Seidman CE.** Constitutively active AMP kinase mutations cause glycogen storage disease mimicking hypertrophic cardiomyopathy. *J Clin Invest* 109: 357-362, 2002.

3. **Banerjee SK, Ramani R, Saba S, Rager J, Tian R, Mathier MA, Ahmad F.** A PRKAG2 mutation causes biphasic changes in myocardial AMPK activity and does not protect against ischemia. *Biochem Biophy Res Commu* 360: 381–387, 2007.

4. **Blair E, Redwood C, Ashrafian H, Oliveira M, Broxholme J, Kerr B, Salmon A, Ostman-Smith I, Watkins H.** Mutations in the gamma(2) subunit of AMP-activated protein kinase cause familial hypertrophic cardiomyopathy: evidence for the central role of energy compromise in disease pathogenesis. *Hum Mol Genet* 10: 1215-1220, 2001.

5. **Bos JM, Towbin JA, Ackerman MJ.** Diagnostic, prognostic, and therapeutic implications of genetic testing for hypertrophic cardiomyopathy. *J Am Coll Cardiol* 54: 201–211, 2009.

6. **Daniel T, Carling D**. Functional analysis of mutations in the gamma 2 subunit of AMP-activated protein kinase associated with cardiac hypertrophy and Wolff-Parkinson-White syndrome. *J Biol Chem* 277: 51017–51024, 2002.

7. **Green EM, Wakimoto H, Anderson RL, Evanchik MJ, Gorham JM, Harrison BC, Henze M, Kawas R, Oslob JD, Rodriguez HM, Song Y, Wan W, Leinwand LA, Spudich JA, McDowell RS, Seidman JG, Seidman CE.** A small-molecule inhibitor of sarcomere contractility suppresses hypertrophic cardiomyopathy in mice. *Science* 351: 617-621, 2016.

8. **Hardie DG, Hawley SA**. AMP-activated protein kinase: the energy charge hypothesis revisited. *Bioessays* 23: 1112-1119, 2001.

9. **Hawley SA, Davison M, Woods A, Davies SP, Beri RK, Carling D, Hardie DG**. Characterization of the AMP-activated protein kinase kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. *J Biol Chem* 271: 27879-27887, 1996.

10. **Hodatsu A, Konno T, Hayashi K, Funada A, Fujita T, Nagata Y, Fujino N, Kawashiri M, Yamagishi M**. Compound heterozygosity deteriorates phenotypes of hypertrophic cardiomyopathy with founder MYBPC3 mutation: evidence from patients and zebrafish models *Am J Ph* 307: H1594-H1604, 2014.

11. **Ingles J, Sarina T, Yeates L, Hunt L, Macciocca I, McCormack L, Winship I, McGaughran J, Atherton J, Semsarian C**. Clinical predictors of genetic testing outcomes in hypertrophic cardiomyopathy. *Genet Med* 15: 972–977, 2013.

12. **Lopes LR, Syrris P, Guttmann OP, O'Mahony C, Tang HC, Dalageorgou C, Jenkins S, Hubank M, Monserrat L, McKenna WJ, Plagnol V, Elliott PM.** Novel genotype-phenotype associations demonstrated by high-throughput sequencing in patients with hypertrophic cardiomyopathy. *Heart* 101: 294-30, 2015.

13. **Luptak I, Shen M, He H, Hirshman MF, Musi N, Goodyear LJ, Yan J, Wakimoto H, Morita H, Arad M, Seidman CE, Seidman JG, Ingwall JS, Balschi JA, Tian R.** Aberrant activation of AMP-activated protein kinase remodels metabolic network in favor of cardiac glycogen storage. *J Clin Invest* 117:1432–1439, 2007.

14. **Maron BJ, Gardin JM, Flack JM, Gidding SS, Kurosaki TT, Bild DE.** Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA Study. Coronary Artery Risk Development in (Young) Adults. *Circulation* 92:785-789, 1995.

15. **Maron BJ, Doerer JJ, Haas TS, Tierney DM, Mueller FO**. Sudden deaths in young competitive athletes: analysis of 1866 deaths in the United States, 1980-2006. *Circulation* 119: 1085–1092, 2009.

16. **Mickelson AV, Gollapudi SK, Chandra M.** Cardiomyopathy-related mutation (A30V) in mouse cardiac troponin T divergently alters the magnitude of stretch activation in α- and β-myosin heavy chain fibers. *Am J Physiol* 312: H141-H149, 2017.

17. **Niimura H, Bachinski LL, Sangwatanaroj S, Watkins H, Chudley AE, McKenna W, Kristinsson A, Roberts R, Sole M, Maron BJ, Seidman JG, Seidman CE**. Mutations in the gene for cardiac myosin binding protein C and late-onset familial hypertrophic cardiomyopathy. *N Engl J Med* 338: 1248–1257, 1998.

18. **Ostman-Smith I, Wettrell G, Keeton B, Riesenfeld T, Holmgren D, Ergander U.** Echocardiographic and electrocardiographic identification of those children with hypertrophic cardiomyopathy who should be considered at high-risk of dying suddenly. *Cardiol Young* 15: 632–642, 2005.

19. **Pause A, Belsham GJ, Gingras AC, Donzé O, Lin TA, Lawrence JC Jr, Sonenberg N**. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function. *Nature* 371: 762-767, 1994.

20. **Rapezzi C, Arbustini E, Caforio AL, Charron P, Gimeno-Blanes J, Heliö T, Linhart A, Mogensen J, Pinto Y, Ristic A, Seggewiss H, Sinagra G, Tavazzi L, Elliott PM**. Diagnostic work-up in cardiomyopathies: bridging the gap between clinical phenotypes and final diagnosis. A position statement from the ESC Working Group on Myocardial and Pericardial Diseases. *Eur Heart J* 34:1448-1458, 2013.

21. **Roma-Rodrigues C, Fernandes AR**. Genetics of hypertrophic cardiomyopathy:

advances and pitfalls in molecular diagnosis and therapy. *Appl Clin Genet* 7: 195-208, 2014.

22. **Shah M**. Hypertrophic cardiomyopathy. *Cardiol Young* 27(S1): S25-S30, 2017.

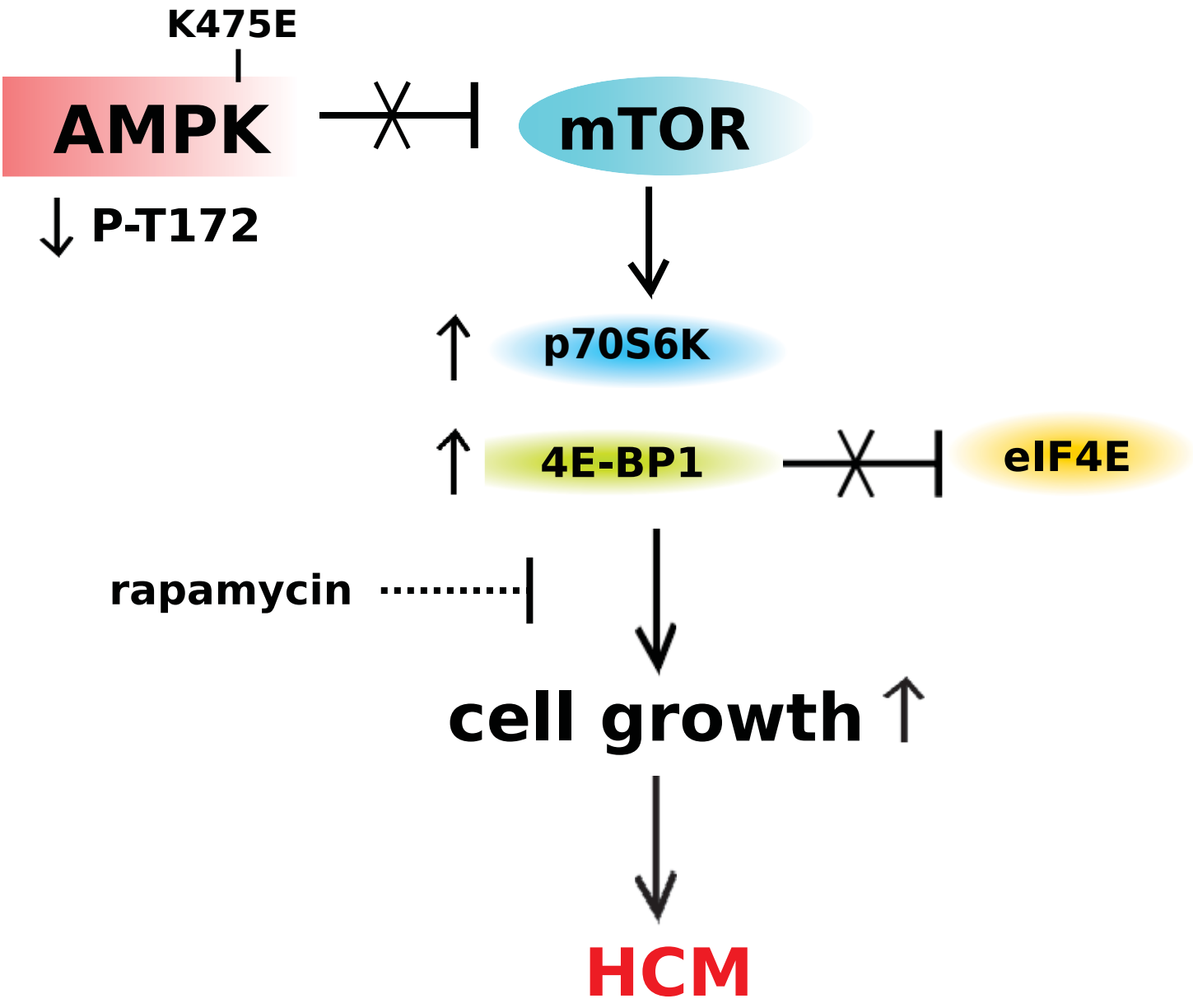
23. **Sidhu JS, Rajawat YS, Rami TG, Gollob MH, Wang Z, Yuan R, Marian AJ, DeMayo FJ, Weilbacher D, Taffet GE, Davies JK, Carling D, Khoury DS, Roberts R.** Transgenic mouse model of ventricular preexcitation and atrioventricular reentrant tachycardia induced by an AMP-activated protein kinase loss-of-function mutation responsible for Wolff-Parkinson-White syndrome. *Circulation* 111: 21-29, 2005.

24. **Wolf CM, Arad M, Ahmad F, Sanbe A, Bernstein SA, Toka O, Konno T, Morley G, Robbins J, Seidman JG, Seidman CE, Berul CI.** Reversibility of PRKAG2 glycogen-storage cardiomyopathy and electrophysiological manifestations. *Circulation* 117: 144-154, 2008.

25. **Xu Y, Gray A, Hardie DG, Uzun A, Shaw S, Padbury JF, Phornphutkul C, Tseng YT**. A novel, de novo mutation in PRKAG2 gene: infantile-onset phenotype and signaling pathway involved. *Am J Physiol* Epub ahead of print, 2017.

26. **Zhang BL, Xu RL, Zhang J, Zhao XX, Wu H, Ma LP, Hu JQ, Zhang JL, Ye Z, Zheng X, Qin YW.** Identification and functional analysis of a novel PRKAG2 mutation responsible for Chinese PRKAG2 cardiac syndrome reveal an important role of non-CBS domains in regulating the AMPK pathway. *J Cardiol* 62: 241-248, 2013.

27. **Zhang L, He H, Balschi JA.** Metformin and phenformin activate AMP-activated protein kinase in the heart by increasing cytosolic AMP concentration. *Am J Physiol* 293: H457-466, 2007.


K475E
AMPK
mTOR
P-T172
p70S6K
4E-BP1
eIF4E
rapamycin
cell growth
HCM