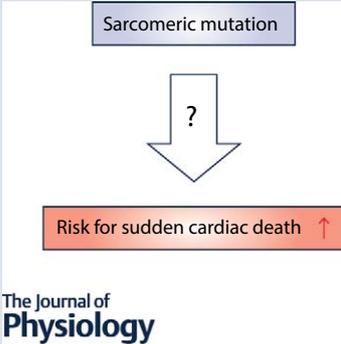


TOPICAL REVIEW

Linking myofilaments to sudden cardiac death: recent advances

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The diagram consists of a white rectangular box on a light blue background. At the top of the box is a blue-bordered rectangle containing the text 'Sarcomeric mutation'. Below this is a large, hollow downward-pointing arrow with a question mark '?' inside it. At the bottom of the box is a red-bordered rectangle containing the text 'Risk for sudden cardiac death' followed by a red upward-pointing arrow '↑'. Below the box, the text 'The Journal of Physiology' is displayed in a serif font.

Abstract The major goal of this focused review is to highlight some of the recent advances and remaining open questions about how a mutation in a myofilament protein leads to an increased risk for sudden cardiac death (SCD). The link between myofilaments and SCD has been known for over 25 years, but identifying mutation carriers at risk for SCD is still a challenge and currently the only effective prevention is implantation of a defibrillator (ICD). In addition to recognized risk factors, other contributing factors need to be considered and assessed, e.g. ‘microvascular dysfunction’, to calibrate individual risk more accurately. Similarly, improving our understanding about the underlying mechanisms of SCD in patients with sarcomeric mutations will also allow us to design new and less invasive treatment options that will minimize risk and hopefully make implantation of an ICD unnecessary.

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Corresponding author S. Huke: Department of Medicine, Division of Cardiovascular Disease, University of Alabama at Birmingham, Birmingham, AL, USA. Email: hukes@uab.edu**Abstract figure legend** This focused review explores recent advances in understanding how mutations in myofilament proteins promote arrhythmias and sudden cardiac death.**Abbreviations** ACCF, American College of Cardiology Foundation; ADP, adenosine diphosphate; AHA, American Heart Association; ATP, adenosine triphosphate; Dip-MBF, myocardial blood flow following dipyridamole infusion; ESC, European Society of Cardiology; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter defibrillator;

Dr Sabine Huke received her PhD in Germany and then pursued post-doctoral opportunities in the US. Her research focuses on mechanisms of sudden cardiac death (SCD) as a result of inherited and acquired conditions. Specifically, how mutations in myofilament proteins cause arrhythmias is one of her major interests since she was awarded an AHA Scientist Development Grant in 2009. Ongoing NIH funded studies examine a link between sarcomeric mutations and coronary vascular dysfunction using mouse models. Currently Dr Huke is at the University of Alabama at Birmingham in the Department of Medicine, Division of Cardiovascular Disease.



LV, left ventricle; LVOTO, left ventricular outflow tract obstruction; MyBPC, myosin binding protein C; PKA, protein kinase A; PKC, protein kinase C; ROS, reactive oxygen species; SCD, sudden cardiac death.

Introduction

Striated muscle contains the 'contractile machinery', the myofilaments, to shorten upon excitation and produce force. The basic unit of the myofilaments is called a 'sarcomere'. Sarcomere function is finely tuned by various intrinsic mechanisms that modulate the dynamics of contraction and relaxation (Kobayashi & Solaro, 2005). The first report of a disease causing mutation in a cardiac sarcomeric protein was published in 1990 (Geisterfer-Lowrance *et al.* 1990). The study discovered that a missense mutation in beta myosin heavy chain formed the molecular basis for an inherited cardiomyopathy termed 'hypertrophic cardiomyopathy' (HCM). Current information links a mutation in a sarcomeric protein with more than 50% of all HCM cases (>1400 mutations in >8 genes known; Maron *et al.* 2012). Most mutation carriers live normal lives often without the requirement for any treatment. However, the disease has a spectrum of clinical presentation and by far the most drastic complication is unexpected sudden cardiac death (SCD). More than a quarter of a century after the first report about the genetic basis for HCM, we are still trying to understand the molecular sequence of events that links a mutation in the myofilaments to an increased risk for SCD.

SCD risk and SCD risk stratification

According to the 2011 ACCF/AHA and the 2014 ESC guidelines, the most reliable predictors for SCD are family history and prior non-lethal events like unexplained syncope and documented arrhythmias (Gersh *et al.* 2011; Elliott *et al.* 2014; Sen-Chowdhry *et al.* 2016). Even though these factors are used to evaluate future SCD risk, they are a marker, but not a cause in itself. Other factors that actually contribute to the risk are structural factors. For example, the degree of left ventricular (LV) hypertrophy has predictive value and recently the left atrial diameter was recognized as a significant factor (see Fig. 1). A relationship between risk and the age of the mutation carrier has been established, i.e. SCD risk seems highest in young subjects and declines with age (Spirito *et al.* 2009; O'Mahony *et al.* 2014).

If an individual demonstrates several of the established risk factors, especially at a young age, this mutation carrier has an elevated risk for SCD and is a candidate for an implantable cardiac defibrillator (ICD). However, most cases are less clear and those are the situations where there is room for improvement. Some patients who received an ICD never experience an appropriate ICD discharge and patients without risk factors can experience

cardiac events (Spirito *et al.* 2014). For example, a recent report describes the case of a teenager who had zero risk factors, but needed to be resuscitated in the school hallway (Kohorst *et al.* 2014). A case like this demonstrates that the structural manifestations observed in HCM cannot be the only reason for the increased SCD risk and we need to evaluate other factors that may contribute as well. Figure 1 groups additional candidate factors that are currently not considered, maybe because they are difficult to measure (e.g. lifestyle) and/or their relevance is not yet understood (e.g. microvascular dysfunction). How 'lifestyle' may affect the natural history of the disease is emphasized by a recent report linking obesity with increased hypertrophy and accelerated progression to heart failure in HCM, while no conclusions about SCD risk were specified (Olivotto *et al.* 2013). Similarly, a typically benign mutation in the myosin light chain shows high penetrance for hypertrophy when hypertension and/or obesity is also present (Claes *et al.* 2016). Understanding more about the mechanism of SCD in subjects with a sarcomeric mutation will not only allow us to determine who should receive an ICD, but will also guide the design of new treatment options that one day may allow us to prevent SCD without the need for an ICD.

Are all sarcomeric mutations the same?

To date, it does not appear that the affected gene predicts which complications to expect over the lifetime of the mutation carrier. Even though HCM is the most common genetic heart disease, finding sufficient numbers of patients to power such studies is still difficult. However, some associations have been established, like the age of onset of ventricular hypertrophy. In general, it is to be expected that mutations in beta myosin heavy chain have early onset, mutations in troponin T intermediate onset and mutations in myosin-binding protein C late onset of LV hypertrophy (Niimura *et al.* 1998). With regard to SCD, the affected gene is currently not considered for risk stratification. An exception may be if multiple pathological mutations are found or the patient is homozygous for a particular mutation, as both have been linked to more severe hypertrophy and higher incidence of SCD (Kelly & Semsarian, 2009). For example, being homozygous for truncated myosin binding protein C (MyBPC) leads to SCD very early in life (Richard *et al.* 2003), which is similarly observed when carrying two different heterozygous mutations in MyBPC (Lekanne Deprez *et al.* 2006). Several earlier studies have raised the notion that mutations in the thin filament, maybe specifically troponin T, are associated with a high incidence of SCD in patients under the age of 45 (Watkins *et al.* 1995;

Moolman *et al.* 1997; Varnava *et al.* 1999). Later studies did not reveal a gene group effect on patient phenotype and the issue is unresolved (Ackerman *et al.* 2002; Pasquale *et al.* 2012). Recently a more comprehensive study compared the clinical presentation and outcome of patients with thin *versus* thick filament mutations (Coppini *et al.* 2014). Systolic dysfunction seemed to be more prevalent in thin filament mutation carriers, but the rate for malignant arrhythmias was not different over an average follow-up period of 4.7 years (SCD, resuscitated cardiac arrest and appropriate ICD shocks). Does this mean this issue is settled? We have to consider that the average age at enrollment in the thin filament group was 44 years of age, so it is possible that the window of maximal risk had already closed and the study included the 'survivors'. Considering that the family history of sudden cardiac death was significantly stronger in the thin filament group (36% *vs.* 18% in thin *vs.* thick filament, respectively), additional studies comparing young mutation carriers may still be warranted.

Vascular involvement

HCM is a very complex disease and despite the fact that the mutant gene is, with few exceptions,

exclusively expressed in cardiomyocytes, the disease has a vascular component (Timmer & Knaapen, 2013; Tardiff *et al.* 2015). Functional parameters most often used to characterize the vascular 'defect' are the maximum myocardial blood flow following dipyridamole infusion (Dip-MBF) or the failure to appropriately raise blood pressure during exercise (abnormal blood pressure response). What has also been described, but is not routinely evaluated, are stress-induced reversible myocardial perfusion defects in HCM patients during exercise. The observed vascular dysregulations fall into the spectrum of 'microvascular dysfunction', but when and why it occurs and how it contributes to SCD risk is not fully understood.

A low Dip-MBF was observed in young HCM patients as soon as they were included in the study (age 15), but study subjects were symptomatic (e.g. dyspnoea and angina) and a larger degree of hypertrophy was already present (Olivotto *et al.* 2011). Hypertrophy and hypoperfusion seem to be logically linked as the cardiomyocyte-to-capillary ratio becomes lower with increasing diameter of the cardiomyocytes. This does not, however, allow the assumption that hypertrophy is always associated with reduced perfusion reserve. The same study found that microvascular function

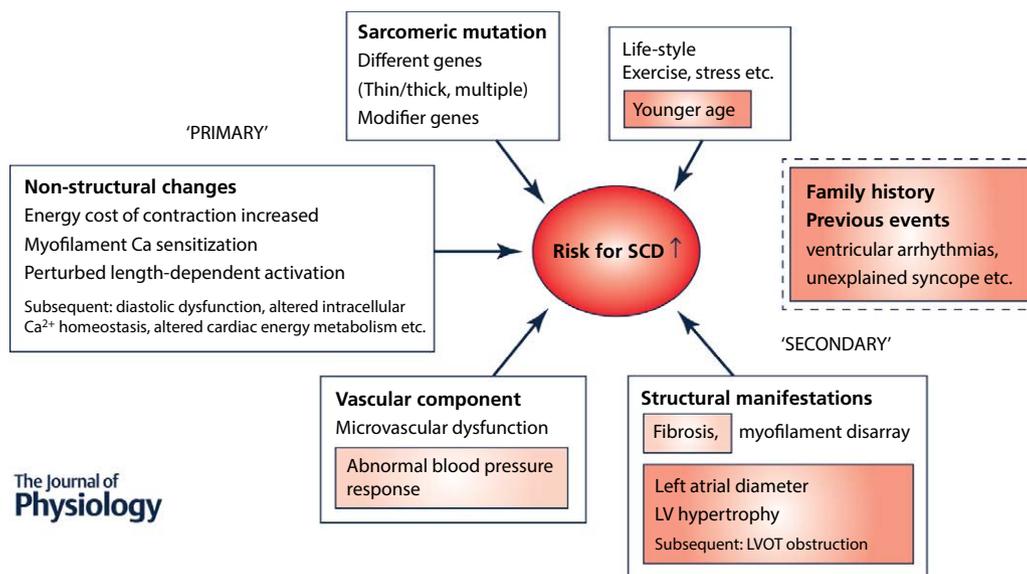


Figure 1. The scheme summarizes factors that are likely to contribute to the increased risk for SCD in HCM

The molecular cause for the SCD risk is the *sarcomeric mutation* which subsequently causes the *non-structural changes* ('primary'). The risk is further modified by lifestyle and the broader genotype of the mutation carrier. Over the lifetime, *structural manifestations* may or may not develop in varying degree and are therefore 'secondary' changes. HCM phenotype clearly has a *vascular component*, but when and why these changes occur is not really understood. SCD risk is most frequently calculated using age and structural parameters, but the most reliable predictors are family history and previous non-lethal events (red background). Other factors are used irregularly by some groups (shaded red background). All contributing factors are interdependent and highly interlinked, while the separation between 'primary' and 'secondary' is often blurry. It would be desirable to base SCD risk and lifestyle recommendations dependably on primary factors rather than to monitor for cardiac remodelling and wait for life-threatening events to occur.

was selectively impaired in patients with sarcomeric mutations, but was typically normal in sarcomere mutation negative patients, despite the presence of hypertrophy. This implies a causal link between myofilaments and microvascular dysfunction that needs to be further explored.

Fixed as well as reversible regional perfusion defects have been reported many times since the 1980s (e.g. O'Gara *et al.* 1987). These earlier studies were usually performed in symptomatic patients with significant hypertrophy and fibrosis; therefore, no conclusions can be drawn about whether the perfusion defects precede the structural changes or are the result. A Swedish group recently reported that while baseline perfusion is compromised in fibrotic regions, hypoperfused regions emerged in hypertrophic tissue adjacent to fibrotic patches during stress perfusion induced with adenosine (Jablonowski *et al.* 2015). The authors concluded that hypoperfusion precedes fibrosis and may be a more sensitive marker of diseased myocardium. The same study included subjects 'at risk' of HCM who were asymptomatic and without hypertrophy, and in that group myocardial perfusion was similar to controls. However, if subjects in the 'at risk' group were all genotype positive and if anyone actually developed the disease at a later age is unknown. Interestingly, the same group also reported that peripheral microvascular function is altered in young 'at risk' subjects. They showed that the response to acetyl choline on forearm perfusion was enhanced, even when no myocardial hypertrophy was evident (Fernelund *et al.* 2015).

How is the 'vascular component' related to prognosis? A lower global Dip-MBF and a lower lateral wall Dip-MBF were associated with adverse cardiovascular events, although this did not include SCD (Castagnoli *et al.* 2016). The only parameter that has been utilized for SCD risk stratification in the past is an abnormal blood pressure response. A study comprising 126 patients showed that while the positive predictive accuracy for cardiovascular mortality was low, the negative predictive accuracy was high, i.e. if your blood pressure response was normal, chances for an adverse cardiovascular event were low (Olivotto *et al.* 1999). However, the recently updated ESC guidelines did not include an abnormal blood pressure response in their risk calculation, due to insufficient statistical evidence. Thus, to date a link between the vascular dysfunction and SCD risk in HCM is not very robust. What needs to be considered though is that SCD in the general population is firmly linked to myocardial perfusion issues, taking into account that coronary artery disease is present in more than 80% of subjects that experience SCD (Myerburg *et al.* 1997). Therefore, it would be premature to not further investigate a potential link between vascular dysfunction and SCD risk in HCM.

Primary, non-structural changes

HCM patients with homozygous or multiple pathological mutations are typically diagnosed younger, have more severe LV hypertrophy and higher SCD risk (see above). This would indicate a dose effect, but a dose effect of what? As it stands, defining the myofilament properties that are commonly altered by sarcomeric mutations, it likely comes down to three options: (1) increased energy cost of contraction, (2) myofilament Ca^{2+} sensitization and (3) perturbed length dependent activation. Of note, how energetics and myofilament Ca sensitivity may facilitate SCD has been reviewed previously in great detail (Huke & Knollmann, 2010). Please refer to the basic mechanisms in this earlier review, as only some recent developments in the field will be discussed here.

Energy cost of contraction increased. An altered energy homeostasis in patients with advanced disease has been unquestionably shown with ever more sophisticated imaging techniques (Shivu *et al.* 2010). Moreover, evidence is accumulating that the impaired energy metabolism occurs early and is potentially a primary effect of the sarcomeric mutation, e.g. it seems to precede the development of hypertrophy (Crilly *et al.* 2003) and contractile abnormalities (Abraham *et al.* 2013). Pre-hypertrophic genotype positive subjects showed decreased myocardial external efficiency, i.e. the performed work per unit of oxygen was lower (Witjas-Paalberends *et al.* 2014). How sarcomeric mutations impair energetics and how that may affect cardiac action potential and facilitate arrhythmias has been discussed previously (Huke & Knollmann, 2010). If and how measures of energy metabolism like the phosphocreatine/ATP ratio or myocardial external efficiency are related to SCD risk has not been tested; in fact the prognostic relevance for any aspect of the disease is only speculative (Timmer & Knaapen, 2013). A role for an energetic impairment may explain why SCD in HCM often occurs during exercise, as the imposed increase in cardiac output further exacerbates the pre-existing energetic deficit (Dass *et al.* 2015).

ADP accumulation during periods when ATP demand exceeds supply may increase myocardial stiffness and contribute to impaired myocardial relaxation and diastolic dysfunction (Sequeira *et al.* 2015a,b). This in turn would increase diastolic LV filling pressure which limits sub-endocardial perfusion, further enhancing an energetic deficit.

It is also possible that a high energy demand from the myofilaments not only leads to an energetic deficit, but has additional consequences. A recent study showed that to support NADH and ATP production in the heart during pathological stress the mitochondrial transhydrogenase (Nnt) changes into reverse-mode and consumes NADPH

instead of producing it (Nickel *et al.* 2015). Since NADPH is necessary, for example, for the reduction of oxidized glutathione, as a consequence the antioxidative capacity may be reduced with the potential for increased oxidative damage.

Myofilament Ca²⁺ sensitization. A second commonly altered myofilament property in HCM is that the myofilament Ca²⁺ sensitivity is increased, i.e. the force–pCa relationship is shifted towards lower [Ca²⁺]. Interestingly, it has been proposed that a differential effect of thin filament mutations on myofilament Ca²⁺ sensitivity is associated with distinct phenotypes: The myofilament Ca²⁺ sensitivity is increased by mutations causing HCM and restrictive cardiomyopathy (RCM), while mutations that decrease myofilament Ca²⁺ sensitivity are associated with inherited forms of dilated cardiomyopathy (DCM) (Willott *et al.* 2010). A recent Dutch study demonstrated that myofilament Ca²⁺ sensitivity is universally increased in human HCM samples with thick and thin filament mutations (Sequeira *et al.* 2013). In several instances though myofilament Ca²⁺ sensitivity was normalized by PKA phosphorylation and thus appeared to be a secondary adjustment rather than a primary effect of the mutant protein itself. It is unclear if the cause for the increase in myofilament Ca²⁺ sensitivity is of importance for the downstream effects.

A link between myofilament Ca²⁺ sensitization and cardiac arrhythmia susceptibility has been systematically tested in mice (Baudenbacher *et al.* 2008). The incidence of ventricular tachycardia during pacing stress was increased in mice with Ca²⁺ sensitizing troponin T mutations or in control hearts after treatment with the Ca²⁺ sensitizer EMD 57033. This was associated with regional conduction slowing predisposing to conduction block and reentry, but the underlying mechanism was not elucidated. Later we showed using the same tools that all arrhythmia inducing protocols in myofilament Ca²⁺ sensitized hearts were associated with ‘focal energy deprivation’ (Huke *et al.* 2013). The anatomic locations of the affected regions were not predictable and were not limited to sub-endocardial tissue. These energy deprived regions may be responsible for the regional conduction slowing and the generation of a substrate for the observed re-entry arrhythmias when myofilaments are Ca²⁺ sensitized.

In addition, myofilament Ca²⁺ sensitization directly affects intracellular Ca²⁺ homeostasis by increasing global cytosolic Ca²⁺ binding affinity (Schober *et al.* 2012). This is only possible because the myofilaments, or more specifically troponin C, have such a large contribution to global Ca²⁺ buffering in muscle tissue. The increase in the Ca²⁺ buffering affinity causes higher end-diastolic [Ca²⁺] at fast pacing rates. This calcium shifts into the sarcoplasmic reticulum during prolonged diastolic

intervals and promotes post-pause action potential prolongation and afterdepolarization and increases the propensity for ventricular ectopy.

Myofilament Ca²⁺ sensitivity has also been associated with the production of reactive oxygen species (ROS) via generation of stretch (Prosser *et al.* 2011; Miura *et al.* 2015). The ROS production, rather than myofilament Ca²⁺ sensitivity itself, was critical for the velocity of Ca²⁺ waves, which is an *in vitro* parameter that is linked to *in vivo* arrhythmia susceptibility.

Altogether, this demonstrates that myofilament Ca²⁺ sensitization is capable of generating both a myocardial substrate and a trigger for lethal arrhythmias, at least when tested in rodents.

Perturbed length dependent activation. Only recently an elegant study showed that in human HCM the length dependent myofilament activation is universally impaired (Sequeira *et al.* 2013). This effect enhances the Frank-Starling mechanism, which is important for the ability of the heart to adjust contractility in response to changes in ventricular filling pressure. In the normal heart, PKA dependent phosphorylation of Troponin I is required to enhance length dependent activation and it is further modulated by PKC phosphorylation (Wijnker *et al.* 2014a,b). The length dependent myofilament activation was drastically reduced in all HCM samples independently of the specific mutation and it was not possible to correct it by increasing PKA phosphorylation. The compromised Frank-Starling response may result in increased wall stress that promotes cardiac remodelling, as speculated elsewhere (Huke & Knollmann, 2013). If and how this impairment contributes to arrhythmia susceptibility is unclear and specific hypotheses are needed.

Future directions

A variety of aspects contribute to the increased risk of SCD in subjects with sarcomeric mutations and their relationship is likely to be complex. Management of affected HCM patients using non-pharmacological surgical strategies (e.g. myectomy to relieve LVOTO) and ICD implantation has greatly improved, thus providing the expectation of a near-normal life span (Maron *et al.* 2016). However, these techniques are invasive, carry risks and reduce quality of life. Less invasive pharmacological treatments that diminish SCD risk and stop the disease from progressing are desirable.

Future opportunities may lie in targeting primary non-structural changes as well as the vascular component. Current pharmacological treatment utilizes compounds that have mild negative inotropic effects (beta blockers, verapamil) and thus are energy sparing. However, this has not been shown to alter the natural history of the disease.

Potentially promising drugs that affect cardiac metabolism by facilitating glucose utilization are perhexiline and trimetazidine. In symptomatic HCM patients perhexiline has been shown to improve cardiac energetics and to increase exercise capacity (Abozguia *et al.* 2010). The drug just entered Phase 2b clinical trials (ClinicalTrials.gov, identifier: NCT02862600) for treatment of HCM patients. Trimetazidine has potential benefits in patients with heart failure of different aetiologies (Fragasso *et al.* 2006), but data specifically in HCM patients are lacking. It is unclear if either drug changes the natural history of the disease and if they are effective in preventing SCD.

Several proof-of-concept studies in mice may indicate that myofilament Ca^{2+} de-sensitization is an interesting new direction. The myofilament Ca^{2+} de-sensitizer W7 has been shown to reduce end-diastolic pressure in isolated perfused hearts (Thompson *et al.* 2016) and another de-sensitizer, blebbistatin, reduced arrhythmia incidence in isolated perfused infarcted hearts (Venkataraman *et al.* 2013). These two compounds, however, cannot be used clinically due to off target effects. Ranolazine, which has a variety of effects and also de-sensitizes the myofilaments, improved high workload tolerance, but did not reverse cardiac hypertrophy or dysfunction in a MyBPC mutant mouse model (Flenner *et al.* 2016). Interestingly, in a mouse model with a mutation in tropomyosin, reducing oxidative modification of myofilaments by treatment with *N*-acetylcysteine normalized myofilament Ca^{2+} sensitivity and reversed diastolic dysfunction and hypertrophy (Wilder *et al.* 2015).

New treatment avenues need to be explored to be able to assure subjects with sarcomeric mutations that the risk for SCD is minimized.

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Additional information

Competing interests

None declared.

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