Hypertrophic Cardiomyopathy
Cell-to-Cell Imbalance in Gene Expression and Contraction
Force as Trigger for Disease Phenotype Development

Theresia Kraft, Judith Montag, Ante Radocaj, Bernhard Brenner

Hypertrophic cardiomyopathy (HCM) is an inherited cardiac disease with an incidence of about 1 in 500 individuals.1 It is characterized by asymmetrical hypertrophy of the left ventricle in the absence of other causes for hypertrophy. HCM can vary from essentially asymptomatic to highly malignant up to end-stage heart failure or cause life-threatening arrhythmias with sudden cardiac death particularly in young adults. In most familial HCM cases, heterozygous mutations in sarcomeric proteins have been identified as underlying cause. About one third of the patients are heterozygous for mutations in the β-cardiac myosin heavy chain gene MYH7, another third in cardiac myosin-binding protein C (cMyBPC).2 Few mutations were found in nonsarcomeric proteins. Cardiomyocyte and myofibrillar disarray with interstitial fibrosis and hypertrophy are hallmarks of HCM.3 The degree of myocardiial disarray correlates with risk factors for sudden cardiac death,4 and it was suggested that myocyte disarray directly results from functional changes induced by the HCM-related mutations at the sarcomeric level.5

From studies mostly based on animal models or recombinant contractile proteins, it was postulated that HCM-associated mutations generally enhance contractility of the myocardium, whereas mutations with reduced contractility would lead to dilated cardiomyopathy.6–8 Accordingly, the different HCM mutations are thought to lead to the HCM phenotype by increasing calcium sensitivity, contractility, and ATPase activity of the cardiomyocytes.5 Results from our group and others, however, were inconsistent with this hypothesis. Instead, contractility and calcium sensitivity can be enhanced or decreased in HCM.5,6,12 Thus, it is still unclear what triggers development of HCM. On the other hand, trials with new therapeutic approaches for HCM would greatly benefit from detailed knowledge of the disease-causing mechanisms.

Here, we propose an entirely new concept of how different mutations in sarcomeric proteins may lead to the common HCM phenotype of the myocardium, and we provide first evidence for this mechanism from studies on cardiac tissue of HCM patients. On the basis of our previous work, we hypothesize that functional imbalance among individual cardiomyocytes significantly contributes to development of the HCM phenotype.10,11 Within the cellular network of the myocardium, functional imbalance with unequal force generation among adjacent cardiomyocytes will result in distortions of cardiomyocytes and nonmyocyte cells. Some of these cells will be overstretched or distorted by cardiomyocytes with higher force generation that themselves over contract. Such distortions not only could initiate cardiomyocyte and myofibrillar disarray but could also trigger stretch-induced signaling, for example, Tgf-β signaling,14 leading to development of interstitial fibrosis and hypertrophy. Thus, cell-to-cell functional variance may initiate hallmarks of the HCM phenotype.15

To test for functional variance among individual cardiomyocytes of affected HCM patients, we determined changes in contractility resulting from HCM-related β-cardyosin mutations.12 As an example, data from a heterozygous patient with mutation R723G are shown here (Figure). When analyzing, cell-by-cell, forces generated by individual cardiomyocytes at concentrations of free calcium-ions attained during a twitch (eg, 2.5×10−6 mol/L or pCa 5.6; Figure [A] and [B]), we find that on average control cardiomyocytes generate ≈40% of the maximum force generated at saturating calcium concentrations, whereas R723G cardiomyocytes generate only ≈15% of maximum force. This is because of lower calcium sensitivity of the R723G cardiomyocytes.12 Importantly, however, we also find a statistically significantly larger cell-to-cell variation in the forces generated by the individual cardiomyocytes than that seen in cardiomyocytes of control samples. In fact, some R723G cardiomyocytes reach 40% of maximum force, just like controls, whereas other cardiomyocytes reach only <5% of their maximum calcium-activated force, reflecting an ≈10-fold difference in force generation of individual R723G cardiomyocytes at the same calcium concentration (Figure [A]). In contrast, the difference among control cardiomyocytes at this calcium concentration was only ≈1.5-fold (Figure [B]).

We speculated that the large difference in force generation of R723G cardiomyocytes results from a similar extent of cell-to-cell variation in expression of mutant β-cardiac myosin. Because we could not directly test such cell-to-cell variation of the expression of mutant β-myosin at the protein level because of insufficient sensitivity of mass spectrometry, we quantified the expression of mutant MYH7 mRNA in individual
cardiomyocytes of the same patient following our previously published methods. Figure [C] shows a representative gel of a quantitative reverse transcription polymerase chain reaction and subsequent allele-specific restriction digest analysis (Figure [D]) of 4 individual R723G cardiomyocytes in duplicates with bands originating specifically from either mutant (labeled R723G) or wild-type (labeled WT) MYH7 mRNA. For cardiomyocytes 1 and 3, there is essentially no band for mutant mRNA, cardiomyocyte 2 shows bands for both mutant and wild-type mRNA, whereas cardiomyocyte 4 has essentially only the band for mutant mRNA. This clearly indicates a large variability in the expression of mutant and wild-type MYH7 alleles in the individual cardiomyocytes. Quantitative analysis of intensity profiles revealed fractions of mutant mRNA of <0.10, 0.70, <0.10, and >0.95 for cardiomyocytes 1 to 4, respectively. The essentially identical band patterns of the duplicates of each cardiomyocyte demonstrate that the experimental error is much smaller than the observed cell-to-cell variability. Figure [E] shows for 35 individual cardiomyocytes the fraction of mutant R723G mRNA derived from quantitative analysis of restriction digests as shown in Figure [C]. For example, 15 of the 35 cardiomyocytes had a fraction of mutant mRNA ≥0.8 and 5 cardiomyocytes ≤0.2. Overall, the distribution of mutant mRNA in individual cardiomyocytes spans the full range from cardiomyocytes with pure mutant mRNA to cardiomyocytes with essentially only wild-type mRNA. This suggests that the observed cell-to-cell variability of force generation indeed may well result from different levels of mutant β-cardiac myosin (protein) in individual cardiomyocytes.

To address the question of what may be the underlying mechanism for this large variation in mutant MYH7 mRNA and mutant β-myosin protein among individual cardiomyocytes, we hypothesized that random, independent burst-like transcription of the mutant and wild-type MYH7 alleles could yield such a mosaic-like expression. To test this, we set up a quantitative model in which the mutant and wild-type MYH7 alleles are stochastically switched on and off such that mutant and wild-type MYH7 mRNA are synthesized in stochastic bursts (Online Figure IA). The modeling also includes protein synthesis from the mRNA such that we could generate a time course of both mutant and wild-type MYH7 mRNA and β-cardiac myosin using published rate constants for synthesis.
and decay of MYH7 mRNA and β-cardiac myosin. The only variables to fit the model output to our experimental data were the rate constants for the stochastic on/off switching of the 2 alleles. Online Figure IA shows the model output with the time courses of the stochastic on/off switching of the 2 alleles, the resulting time courses and fractions of mutant and wild-type MYH7 mRNA and β-cardiac myosin, respectively. To obtain values for individual sample cardiomyocytes, the fractions of mutant MYH7 mRNA and mutant protein were selected from independent points of the simulated time course, each representing a sample cardiomyocyte. The predicted fraction of mutant mRNA for a large population of sample cardiomyocytes is shown in Online Figure IB.

The fraction of mutant β-cardiac myosin was then used to predict the force levels expected to be generated by the sample cardiomyocytes at partial activation (pCa 5.6; Online Figure IC). Assuming the reduction in calcium sensitivity seen experimentally for R723G cardiomyocytes at pCa 5.6 to be proportional to the fraction of expressed mutant β-cardiac myosin, we could calculate for each sample cardiomyocyte from the predicted fraction of mutant β-cardiac myosin the expected force at partial activation at pCa 5.6 (Online Figure IC). Note that both plots, Online Figure IB and IC, are similar to the corresponding data shown in Figure [A] and [E]. All these were achieved with MYH7 alleles being randomly switched on and off. Preliminary evidence from visualizing pre-mRNA in active transcription sites by fluorescence in situ hybridization, in fact, revealed a substantial fraction of nuclei without active transcription sites, just as expected from stochastic, burst-like transcription of mutant and wild-type MYH7 alleles (data not shown).

Essential points of our concept for the development of typical HCM features are (1) that most HCM-related mutations are heterozygous and are found in proteins where they may directly alter mechanical properties of the cardiomyocytes and (2) that mutant and wild-type alleles are not equally expressed at any given time point. Thus, if a HCM mutation alters contractility of the sarcomere, the unequal expression from cell to cell, which we found for the 2 alleles of MYH7, will result in contractile heterogeneity among the tightly interconnected neighboring cardiomyocytes during each heartbeat. In the long run, distortions of cardiomyocytes and nonmyocytes in such a functional mosaic may well lead to cardiomyocyte disarray and trigger interstitial fibrosis, thus severely impairing normal myocardial function. The phenotype certainly is modulated by several other factors, including the direct functional impact of the mutation on the respective protein. In this concept, it is well conceivable that therapeutic interventions that reduce cardiomyocyte force generation and thus reduce functional imbalances can result in a milder phenotype and delay early left ventricular remodeling, just as interventions that inhibit Tgf-β signaling by distorted cardiomyocytes and nonmyocytes may delay development of fibrosis.

Clearly, further studies are needed to test this concept by, for example, following functional imbalance from early onset of the disease and by identifying signaling paths triggered by the distortions of cardiomyocytes and noncardiomyocytes. Because such studies are difficult with human tissue samples, cellular models of cardiomyocytes derived from affected patients via induced pluripotent stem cells or suitable, preferably large-animal models are required. In addition, such measurements should be extended to other myosin mutations and mutations in other sarcomeric and nonsarcomeric proteins. We would not be surprised if a mutation in any protein, sarcomeric or nonsarcomeric, that increases or decreases force generation in cardiomyocytes will result in similar cell-to-cell functional imbalance, if expression of the mutant and wild-type alleles varies from cell to cell, for example, by independent, stochastic, burst-like transcription.

Sources of Funding

This work was supported by a HiLF-Grant (Hochschulinterne Leistungsförderung) of Hannover Medical School to J. Montag, by a grant of the Braumann-Wittenberg-Herz-Stiftung, and by grants of the German Research Foundation (DFG) to T. Kraft (KR1187/19-1 and KR1187/22-1).

Disclosures

None.

References


Key Words: allelic imbalance ■ contractile protein ■ cardiomyocytes ■ heart failure ■ hypertrophic cardiomyopathy ■ myosin heavy chains
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Circ Res. 2016;119:992-995
doi: 10.1161/CIRCRESAHA.116.309804

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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Hypertrophic cardiomyopathy
Cell-to-cell imbalance in gene expression and contraction force
as trigger for disease phenotype development

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Supplemental Material
Online Figure I
Online Figure I: Predictions from model calculations of independent, stochastic, burst-like transcription of mutant and wildtype MYH7-alleles. A, Sample time course of stochastic on/off switching of transcriptions sites, mutant (red trace) and wildtype (black trace) MYH7-mRNA, fraction of mutant MYH7-mRNA, and fraction of mutant β-cardiac myosin predicted by quantitative modelling assuming stochastic, independent on/off switching of the mutant and wildtype MYH7-alleles. With rate constants for mRNA and protein synthesis and decay taken from the literature, only kinetics for on/off switching were adjustable variables in the modelling. To account for the average mutant MYH7-mRNA fraction of 0.7 measured in R723G tissue, the decay rate of mutant MYH7-mRNA was set to half of the decay rate of wildtype MYH7-mRNA. B, Distribution of fraction of mutant mRNA in individual sample-cardiomyocytes predicted by model (cf. Fig. 1E for experimental data). C, Forces at partial activation (pCa 5.6) predicted from fraction of mutant β-cardiac myosin at 35 independent time points of sample run as shown in (A). Dash-dot line, mean value, dashed lines delineate mean ± 1.96SD. Note similarity to data shown in Fig. 1A.

Supplemental References