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Original article

Hypertrophic cardiomyopathy associated Lys104Glu mutation in the myosin regulatory light chain causes diastolic disturbance in mice

Wenrui Huang [a], Jingsheng Liang [a], Katarzyna Kazmierczak [a], Priya Muthu [a], Divya Duggal [b], Gerrie P. Farman [c], Lars Sorensen [d], Iraklis Pozios [d], Theodore P. Abraham [d], Jeffrey R. Moore [c], Julian Borejdo [b], Danuta Szczesna-Cordary [a,*]

[a] University of Miami, Miller School of Medicine, Miami, FL 33136, United States
[b] University of North Texas Health Science Center, Fort Worth, TX 76107, United States
[c] Boston University School of Medicine, Boston, MA 02118, United States
[d] Johns Hopkins University School of Medicine, Baltimore, MD 21205, United States

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ABSTRACT

We have examined, for the first time, the effects of the familial hypertrophic cardiomyopathy (HCM)-associated Lys104Glu mutation in the myosin regulatory light chain (RLC). Transgenic mice expressing the Lys104Glu substitution (Tg-MUT) were generated and the results were compared to Tg-WT (wild-type human ventricular RLC) mice. Echocardiography with pulse wave Doppler in 6 month-old Tg-MUT showed early signs of diastolic disturbance with significantly reduced E/A transmitral velocities ratio. Invasive hemodynamics in 6 month-old Tg-MUT mice also demonstrated a borderline significant prolonged isovolumic relaxation time (Tau) and a tendency for slower rate of pressure decline, suggesting alterations in diastolic function in Tg-MUT. Six month-old mutant animals had no LV hypertrophy; however, at >13 months they displayed significant hypertrophy and fibrosis. In skinned papillary muscles from 5 to 6 month-old mice a mutation induced reduction in maximal tension and slower muscle relaxation rates were observed. Mutated cross-bridges showed increased rates of binding to the thin filaments and a faster rate of the power stroke. In addition, ~2-fold lower level of RLC phosphorylation was observed in the mutant compared to Tg-WT. In line with the higher mitochondrial content seen in Tg-MUT hearts, the MUT-myosin ATPase activity was significantly higher than WT-myosin, indicating increased energy consumption. In the *in vitro* motility assay, MUT-myosin produced higher actin sliding velocity under zero load, but the velocity drastically decreased with applied load in the MUT *vs.* WT myosin. Our results suggest that diastolic disturbance (impaired muscle relaxation, lower E/A) and inefficiency of energy use (reduced contractile force and faster ATP consumption) may underlie the Lys104Glu-mediated HCM phenotype.

1. Introduction

Cardiac muscle contraction relies on the ATP-dependent interaction of the myosin heads (S1) with actin to produce the sliding filament movement and generate force [1]. Myosin S1 consists of two major structural domains: the motor domain with the actin and ATP binding sites, and the lever arm domain composed of an 8.5 nm long α-helical region of the myosin heavy chain (MHC) containing the sites of attachment for the myosin regulatory (RLC) and essential (ELC) light chains [2]. Both light chains support the structure and function of the myosin lever arm and contribute to the power stroke and force generation processes. It is therefore very likely for genetically mutated light chains to induce detrimental effects on the mechanical properties of the lever arm and to compromise the ability of myosin motor to produce force and muscle contraction [3].

The myosin RLC is a member of the EF-hand Ca^{2+} binding protein family, which includes troponin C and calmodulin. Unlike other EF-hands, RLC contains only one Ca^{2+} binding EF-hand domain with the helix-loop-helix motif, which can be occupied by either Ca^{2+} or Mg^{2+} [4] (Fig. 1A). It has been postulated that during cardiac muscle contraction, this site could act as a delayed Ca^{2+} buffer helping the sarcoplasmic reticulum in sequestering Ca^{2+} during diastole [5,6]. Another functionally important domain of the RLC is the myosin light chain

Abbreviations: ACF, auto correlation function; E/A, early (E) to late (A) mitral inflow ratio; EDC, 1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide; EF, ejection fraction; ELC, myosin essential light chain; HCM, hypertrophic cardiomyopathy; LV, left ventricle; Lys104Glu, lysine at position 104 substituted by glutamic acid; MHC, myosin heavy chain; MLCK, myosin light chain kinase; MYL2, human gene encoding the ventricular myosin RLC; NTg, non-transgenic; RLC, myosin regulatory light chain; SCD, sudden cardiac death; Tau, time of isovolumic relaxation; TDI, tissue Doppler imaging; Tg-MUT, transgenic mutant RLC-Lys104Glu; Tg-WT, transgenic wild type RLC; VDAC, voltage dependent anion channel.

* Corresponding author at: University of Miami Miller School of Medicine, 1600 NW 10th Ave, Miami, FL 33136, United States. Tel.: +1 3052432908; fax: +1 3052434555.
E-mail address: dszczesna@med.miami.edu (D. Szczesna-Cordary).

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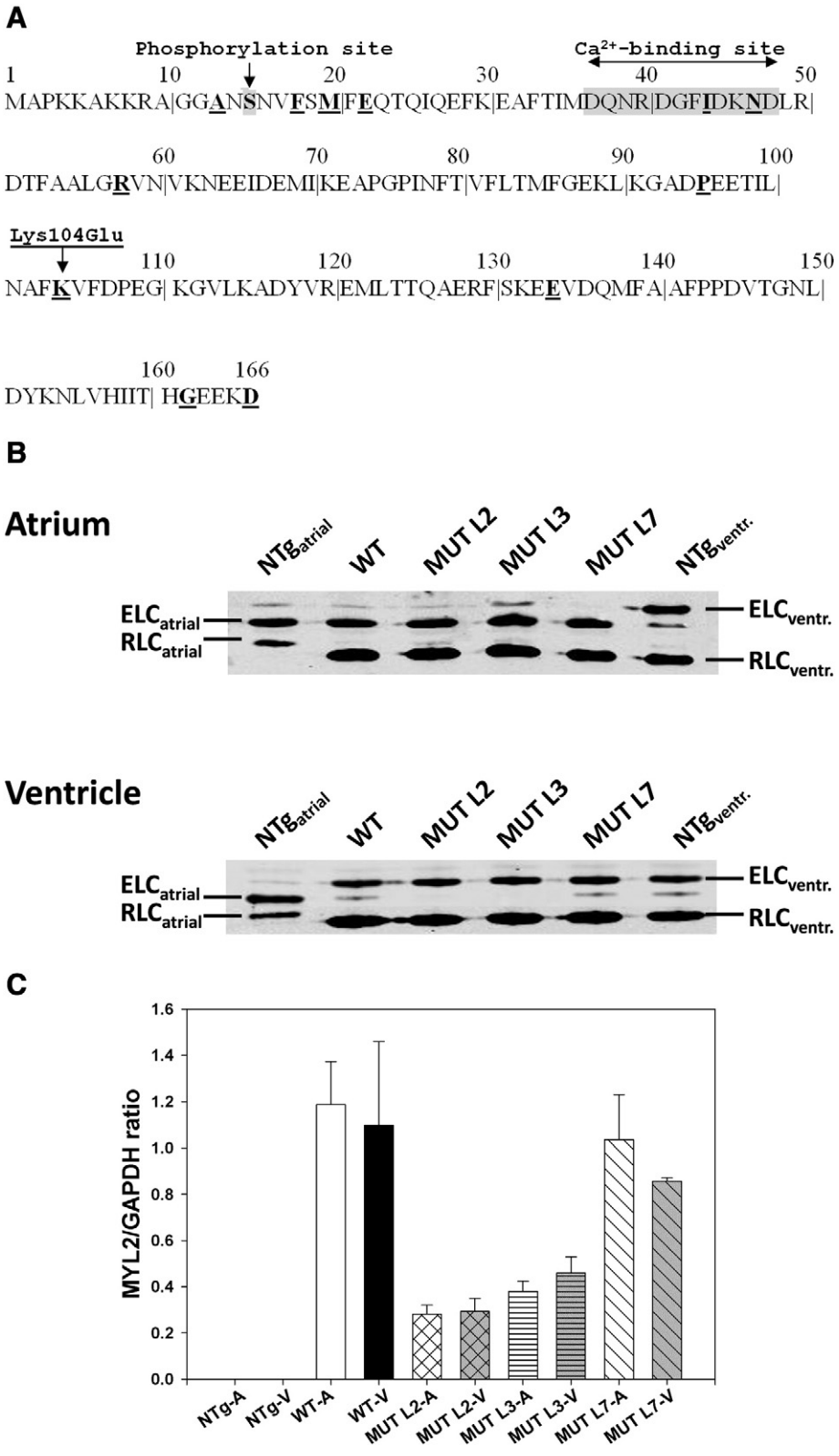

Fig. 1. The Lys104Glu mutation in the human ventricular RLC expressed in transgenic mice. A. Amino acid sequence of the human ventricular myosin RLC (GenBank accession no. P10916) and the sites of HCM-associated mutations (underlined); also indicated the Ser15 phosphorylation site and the Ca^{2+}-binding site (shadowed). B. RLC-Lys104Glu expression in transgenic mice. Atrial (upper panel) and ventricular (lower panel) myofibrils from Tg-MUT L2, L3 and L7 mice were probed for protein expression: 95 ± 2% ($n = 12$) (L2), 97 ± 1% ($n = 12$) (L3) and 99 ± 0.5% ($n = 12$) (L7) of Lys104Glu RLC mutant were incorporated into the hearts of Tg-MUT mice compared to 97.0 ± 3% ($n = 12$) of Tg-WT L2 generated previously [6]. The data are presented as average of *n* experiments performed on cardiac myofibrils and heart extracts ± SEM. No differences in protein expression level were observed between the preparations. Note that $RLC_{ventr.}$ migrated faster than RLC_{atrial} while no differential gel mobility was monitored between the mouse and human $RLC_{ventr.}$. Abbreviations: NTg, non-transgenic; L, mouse line; $RLC_{ventr.}$, $ELC_{ventr.}$, ventricular isoforms of RLC or ELC. C. Real-time PCR quantification of human *MYL2* gene expression in the atria and ventricles of Tg-WT and Tg-MUT L2, L3 and L7 mice. The results were expressed as a ratio of *MYL2* to *GAPDH* (housekeeping gene). Note that similar levels of mRNA expression were observed between atria (A) and ventricles (V) in all tested transgenic lines.