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RAPID COMMUNICATION

A novel mutation of glycogen synthase kinase-3β leads to a reduced level of GSK3β protein in a patient with dilated cardiomyopathy

Dilated cardiomyopathy (DCM) is a frequently inherited heart condition. A familial transmission rate of 20%–35% has been documented, and most of these cases are autosomal dominant. This condition has a high rate of morbidity and mortality, which can lead to cardiac arrest and persistent heart failure.[1] Several biological processes are regulated by the constitutively active serine/threonine protein kinase glycogen synthase kinase-3β (GSK-3β), constitutively active. It is a negative regulator of glucose homeostasis and is involved in energy metabolism, inflammation, endoplasmic reticulum stress, mitochondrial dysfunction, and apoptotic pathways. E-cadherin is repressed in epithelial–mesenchymal transition (EMT), allowing the passage of attached epithelial cells to the mesenchymal state. B-Catenin represses E-cadherin expression to promote EMT. In the WNT pathway, GSK-3β regulates E-cadherin transcription by phosphorylating β-catenin to trigger proteasomal degradation. Although numerous mechanisms remain unknown, GSK-3β has demonstrated a significant role in heart cardiomyocyte differentiation, hypertrophy, and fibrosis.[2] The ability of GSK-3β to control cardiac hypertrophy, which can both cause and treat disease, may have a dosage-dependent impact.[3] Abnormalities in the cardiac Z-disc were found in GSK-3β conditional knockout mice, which affected myocardial contraction.[4] Few people with primary GSK-3β mutations have been identified, and prior research has mostly focused on gene-edited mice or cells. A potentially pathogenic GSK-3β variant was identified in the current study in a patient with DCM, which has significant ramifications for future genetic research on both DCM and GSK-3β.

Here, we report the case of a 30-year-old female patient with DCM and severe heart failure. Three years ago, the patient experienced shortness of breath and chest discomfort following exercise, accompanied by cough and expectoration. She visited our hospital intermittently during this time, and her health was stable. The electrocardiogram results from a recent hospital stay showed (i) sinus rhythm, (ii) little variation in potential, and (iii) enlarged right ventricle. The heart shadow was enlarged on the chest radiograph. Figure 1A shows the results of the echocardiography test: left ventricle, 59 mm; left atrium, 35 mm; right ventricle, 60 mm; right atrium, 64 mm; and ejection fraction (EF), 33%. These results revealed (i) enlargement of the right and left ventricles, (ii) general weakness and lack of coordination in ventricular wall movements, and (iii) left and right ventricular dysfunction during systole. The right heart catheterization pressure analysis showed that the entire lung positive force was 4.79 Wood, the whole lung resistance increased slightly, the pulmonary artery pressure was normal, and the right atrial pressure was elevated. As shown in the cardiac MRI, the anterior and lateral epicardium of the left ventricle displayed delayed enhancement. Right ventricle EF was 7% and left ventricle EF was 9%. Pathological biopsy revealed cardiomyocyte hypertrophy with scattered, enlarged, and deeply stained odd-shaped nuclei, partial cardiomyocyte fibrosis, and adipose tissue infiltration between the cardiac fibers. Clinical signs and other tests were used to diagnose DCM.

For whole-exome sequencing, we collected blood samples from the patient and extracted DNA. We then filtered the data as follows: (i) we ranked genes using Polyphen-2, SIFT, MutationTaster, and CADD; (ii) we excluded non-coding regions and synonymous variants that did not affect splicing; and (iii) we examined variants in the 1000 g, Exome Aggregation Consortium, and gnomAD databases.

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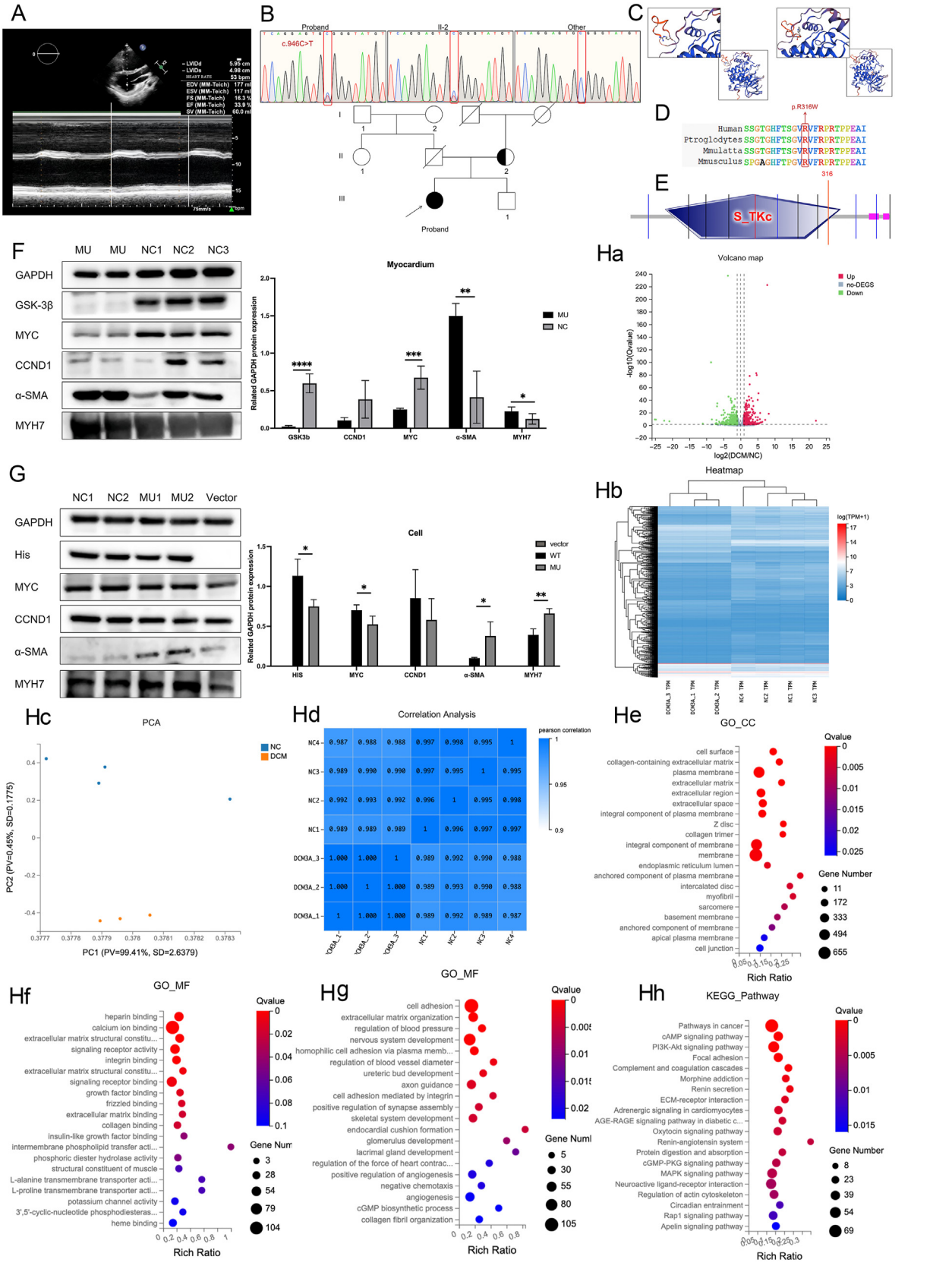

Figure 1 Findings from patient genetic analysis, mutation characterization, clinical images, molecular experiments, and transcriptome sequencing. **(A)** Cardiac ultrasound results. **(B)** Sanger validation of mutations and family tree. **(C)** 3D structure prediction of mutated and normal controls, normal on the left and mutated on the right. **(D)** Conserved assessment of mutated loci. **(E)** Distribution of important structural domains and mutation locations in GSK-3β. **(F)** Western blotting results of human myocardial tissues. **(G)** Western blotting results of transfected AC16 cells. **(H)** Transcriptome sequencing of human myocardial tissues.

Sanger sequencing was used to identify a unique non-synonymous variant [NM 002093.3:c.946C > T:p. R316W] of GSK-3β, which led to the R316W substitution (Fig. 1B). The protein architecture of GSK-3β is displayed. The spatial structures of the wild type (R513) and mutant (W513) were dissimilar (Fig. 1C). The GSK-3β amino acid composition is very conserved (Fig. 1D). The essential structural domain of the gene contains a mutation site (Fig. 1E).

Using the American College of Medical Genetics assessment standards, we thoroughly examined the pathogenicity of this variant and concluded that it is likely pathogenic (PS3 + PM1 + PM2 + PP3). Myocardial tissue was extracted from the left ventricle of three healthy controls and the patient. Additionally, we created vectors containing wild-type and mutant GSK-3β and confirmed them using Sanger sequencing. We also cloned the human GSK-3β gene-coding sequence. Proteins were extracted from the transfected AC16 cardiomyocytes and human left ventricle myocardial tissue from both the wild-type and mutant groups. The findings showed that the GSK-3β/HIS protein level was considerably lower in the mutant group, whereas MYC expression was lower and MYH7 and α-SMA expression was higher in the wild-type group ($P < 0.05$). Additionally, CCND1 expression decreased, but this was not statistically significant (Fig. 1F, G). Western blotting results suggested that GSK-3β mutation likely affects transcription and translation or reduces protein stability, leading to loss of function, which in turn causes alterations in proteins that indicate cardiomyopathy.

To understand the molecular changes and biological processes involved in cardiac tissue alterations, mRNA expression in the mutant and wild-type control myocardial tissue was compared using RNA-seq. In comparison with normal cardiac tissue, 1375 DEGs were identified using the selection criteria in the Methods section (q-value <0.05 and $|\log_2 FC| > 1$), of which 553 were up-regulated, and 822 were down-regulated (Fig. 1H—a). Heatmaps were used to show the DEG expression patterns, and hierarchical clustering analysis was used to highlight how the two groups differed in terms of gene expression (Fig. 1H—b). PCA and correlation analysis were also performed on the samples (Fig. 1H—c, d). The DEGs were subjected to GO function and KEGG pathway enrichment analyses, and the results are depicted in Figure 1H-e—h.

Although the mother of the patient carried the same mutation, mutational penetrance is frequently observed in cardiomyopathy patients. As a result, we attribute this to penetrance and individual differences. Our findings revealed that GSK-3β mutation caused a drop in its expression, which in turn led to several cardiomyopathy symptoms. The genetic landscape of DCM was expanded in this study.

Ethics declaration

The studies involving human subjects were reviewed and approved by the Ethics Committee of the Second Xiangya Hospital of Central South University, Hunan, China.

Conflict of interests

The authors declare no conflict of interests.

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