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GATA4 loss-of-function mutation underlies familial dilated cardiomyopathy ☆

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ABSTRACT

The cardiac transcription factor GATA4 is essential for cardiac development, and mutations in this gene have been implicated in a wide variety of congenital heart diseases in both animal models and humans. However, whether mutated GATA4 predisposes to dilated cardiomyopathy (DCM) remains unknown. In this study, the whole coding region and splice junction sites of the *GATA4* gene was sequenced in 110 unrelated patients with idiopathic DCM. The available relatives of the index patient harboring an identified mutation and 200 unrelated ethnically matched healthy individuals used as controls were genotyped. The functional effect of the mutant GATA4 was characterized in contrast to its wild-type counterpart using a luciferase reporter assay system. As a result, a novel heterozygous GATA4 mutation, p.C271S, was identified in a family with DCM inherited as an autosomal dominant trait, which co-segregated with DCM in the family with complete penetrance. The missense mutation was absent in 400 control chromosomes and the altered amino acid was completely conserved evolutionarily among species. Functional analysis demonstrated that the GATA4 mutant was associated with significantly decreased transcriptional activity and remarkably reduced synergistic activation between GATA4 and NKX2-5, another transcription factor crucial for cardiogenesis. The findings provide novel insight into the molecular mechanisms involved in the pathogenesis of DCM, suggesting the potential implications in the prenatal diagnosis and gene-specific treatment for this common form of myocardial disorder.

1. Introduction

Dilated cardiomyopathy (DCM), the most common form of primary myocardial disorder characterized by ventricular chamber enlargement and contractile dysfunction with normal left ventricular wall thickness, is a major cause of congestive heart failure and sudden cardiac death and is the leading indication for cardiac transplantation in patients worldwide [1]. Approximately 50% of DCM cases are idiopathic, of which 25%–50% are familial, with estimates that vary based on the family members screened [2]. A growing body of evidence demonstrates that genetic risk factors play an important role in the pathogenesis of idiopathic DCM, and a long list of mutations in at least 50 single genes have been linked to familial HCM [2]. Functional characterization of these genetic variations indicates that multiple defects in myocardial structures and cardiac signal molecules may lead to DCM [2]. Despite the seemingly vast number of genes and mutations associated with DCM, the molecular basis for HCM in an overwhelming majority of patients remains unclear.

The zinc-finger transcription factor GATA4 is highly expressed in cardiomyocytes at different developmental stages and continues expression in the adult cardiac myocytes, where it regulates the transcription of several key structural and regulatory genes, including atrial natriuretic factor (ANF), brain natriuretic factor, carnitine palmitoyltransferase Iβ, troponin I, troponin C, α- and β-myosin heavy chain [3–5]. In humans, GATA4 has been demonstrated to be crucial for normal cardiogenesis, as shown by the

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established association of GATA4 mutations with a wide variety of congenital cardiovascular abnormalities, including atrial septal defect (ASD), ventricular septal defect, tetralogy of Fallot, endocardial cushion defect, patent ductus arteriosus, pulmonary stenosis, and hypoplastic right ventricle [6–9]. In mice, homozygous GATA4 deficiency results in early embryonic lethality because of abnormal embryogenesis and heart tube formation [10,11]. In contrast, mice expressing 70% less GATA4 protein died between days 13.5 and 16.5 of gestation, and in these embryos, common atrioventricular canal, double outlet right ventricle and hypoplastic ventricular myocardium were observed [12]. Furthermore, transgenic mice expressing GATA4 mutants showed various cardiac malformations, including septal defects, right ventricular hypoplasia, endocardial cushion defect, tetralogy of Fallot, double outlets of the right ven tricle, and cardiomyopathy, similar to the anomalies seen in humans [8]. More importantly, gene-targeted mice with marked loss of GATA4 protein in the heart survived into adulthood but displayed progressive cardiac enlargement and dysfunction with increased rates of cardiomyocyte apoptosis that was correlated to GATA4 levels [13]. These data highlight the pivotal role of GATA4 in maintaining proper homeostatic remodeling in adult hearts by promoting cell survival and regeneration and inhibiting programmed cell death [14–17].

GATA4 regulates cardiac gene expression by forming complexes with other transcriptional factors, including NKX2-5, TBX5, SRF, SMAD1, SMAD4, and JARID2 [5]. NKX2-5 is another critical regulator of cardiac development with expression and functions that overlap with GATA4 during embryogenesis [9]. Moreover, GATA4 and NKX2-5 physically interact and have been shown to cooperatively regulate the expression of multiple essential cardiac target genes, including those encoding ANF, T- and L-type Ca^{2+} channels, connexin40, α-actin, ID2 and LRRC10 [5]. Targeted knockout of NKX2-5 in mice gives rise to impaired cardiac growth and chamber formation, deranged gene regulatory network, and early embryonic death, while cardiac-specific deletion of NKX2-5 causes progressive cardiomyopathy and complete heart block [1–20]. Mutations in the human NKX2-5 gene have been related to a diverse range of congenital heart diseases, including atrial and ventricular septal defects, tetralogy of Fallot, hypoplastic left heart, transposition of the great arteries, valvular malformations, left ventricular contractile dysfunction, and DCM [21–23]. These results justify screening GATA4 as a prime candidate gene for DCM.

2. Materials and methods

2.1. Study population

A total of 110 unrelated patients with idiopathic DCM were recruited from the Han Chinese population. The available relatives of the index patients were also enrolled. The controls were 200 ethnically-matched unrelated healthy individuals. All participants were evaluated by detailed history, physical examination, chest radiography, electrocardiogram, echocardiography, and exercise performance testing. Cardiac catheterization, angiography, endomyocardial biopsy, and cardiac magnetic resonance imaging were performed only if there was a strong clinical indication. Medical records were also reviewed in the case of deceased or unavailable relatives. Diagnosis of idiopathic DCM was made in accordance with the criteria established by the World Health Organization/International Society and Federation of Cardiology Task Force on the Classification of Cardiomyopathy: a left ventricular end-diastolic diameter >27 mm/m^2 and an ejection fraction <40% or fractional shortening <25% in the absence of abnormal loading conditions, coronary artery disease, congenital heart lesions, and other systemic diseases [24]. Individuals were excluded if they had insufficient echocardiographic image quality, or coexistent conditions that may lead to contractile dysfunction, such as uncontrolled systemic hypertension, coronary artery disease, or valvular heart disease. Familial DCM was defined as having two or more first-degree relatives with idiopathic DCM. Peripheral venous blood specimens from the study subjects and control individuals were prepared. All clinical studies were performed with investigators blinded to the results of genetic testing. This study conformed to the principles of the Declaration of Helsinki and the study protocol was approved by the local institutional ethics committee. Written informed consent was obtained from all participants prior to investigation.

2.2. Genetic studies

Genomic DNA was extracted from blood lymphocytes of all participants with Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The whole coding region and splice junction sites of the *GATA4* gene was sequenced in 110 unrelated patients with idiopathic DCM. Genotyping GATA4 in the available relatives of the proband carrying an identified mutation and 200 unrelated healthy controls was performed. The referential genomic DNA sequence of GATA4 derived from GenBank (accession No. NC_000008). The primer pairs used to amplify the coding exons and intron–exon boundaries of GATA4 by polymerase chain reaction (PCR) were designed as described previously [25]. The PCR was carried out using HotStar Taq DNA Polymerase (Qiagen, Hilden, Germany) on a PE 9700 Thermal Cycler (Applied Biosystems, Foster, CA, USA) with standard conditions and concentrations of reagents. Amplified products were purified with the QIAquick Gel Extraction Kit (Qiagen). Both strands of each PCR product were sequenced with a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) under an ABI PRISM 3130 XL DNA Analyzer (Applied Biosystems). DNA sequences were viewed and analyzed with the DNA Sequencing Analysis Software v5.1 (Applied Biosystems). The variant was validated by resequencing of an independent PCR-generated amplicon from the subject. In addition, for an identified sequence variant, the single nucleotide polymorphism (SNP; http://www.ncbi.nlm.nih.gov/SNP) and Exome Variant Server (EVS; http://evs.gs.washington.edu/EVS) databases were queried to confirm its novelty.

2.3. Multiple sequence alignments

Multiple GATA4 protein sequences across various species were aligned using the online MUSCLE program, version 3.6 (http://www.ncbi.nlm.nih.gov/).

2.4. Plasmids and site-directed mutagenesis

The recombinant expression plasmids GATA4-pSSRa, NKX2-5-pEFSA, and ANF-luciferase reporter (ANF-luc), which contains the 2600-bp 5′-flanking region of the ANF gene, were kindly provided by Dr. Ichiro Shiojima from Chiba University School of Medicine, Japan. The identified mutation was introduced into the wild-type GATA4 using a QuickChange II XL Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) with a complementary pair of primers. The mutant was sequenced to confirm the desired mutation and to exclude any other sequence variations.

2.5. Reporter gene assays

Hela cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. The internal control reporter plasmid pGL4.75 (hRluc/CMV, Promega) were used in transient transfection assays to examine the transcriptional activity of

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