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KLF13 loss-of-function variation contributes to familial congenital heart defects

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Abstract. – OBJECTIVE: Congenital heart defect (CHD) represents the most common form of human developmental abnormality and contributes to substantial morbidity, mortality, and socioeconomic burden worldwide. Accumulating evidence underscores the strong genetic basis of CHD. Nevertheless, CHD is of pronounced genetic heterogeneity, and the genetic determinants underlying CHD in most patients are still unclear. This study was mainly sought to identify the causative gene for CHD in a consanguineous Chinese family.

PATIENTS AND METHODS: Whole-exosome sequencing and bioinformatics analyses were performed in a Chinese family with CHD (double-outlet right ventricle and ventricular septal defect), which was transmitted in an autosomal dominant pattern. A total of 312 unrelated healthy individuals were then genotyped for the identified genetic variation. The functional effect of the identified variation was characterized by utilizing a Dual-Luciferase reporter assay system.

RESULTS: A novel heterozygous variation, NM_015995.3: c.370G>T; p.(Glu124*), was identified in the KLF13 gene, which encodes Kruppel-like factor 13 key to proper heart development. Genetic analysis of the pedigree unveiled that the variation co-segregated with CHD, with complete penetrance. The variation was absent from 624 control chromosomes. The biological analysis revealed that the Glu124*-mutant KLF13 protein failed to transactivate its cardiac target genes ACTC1 and ANP. Furthermore, the variation disrupted the synergistic transactivation between KLF13 and GATA4, as well as GATA6, two other genes that have been recognized to cause CHD.

CONCLUSIONS: These findings firstly indicate that genetically defective KLF13 predisposes to familial CHD, implying potential implications for genetic counseling and an improved prophylactic strategy in a subset of CHD patients.

Key Words:
Congenital heart disease, Molecular genetics, Transcriptional factor, KLF13, Reporter gene assay.

Introduction

Congenital heart defect (CHD), which usually refers to any structural malformation of the heart or endothoracic great blood vessels arising before birth, is the most common birth defect in humans with an estimated 1% prevalence in live births[1]. If minor cardiac anomalies, such as bicuspid aortic valve are included, the total prevalence of CHD is up to 2% to 3%[1]. Although minor CHD can resolve spontaneously[2], major CHD may result in degraded health-related quality of life[3-6], decreased exercise performance[7-10], retarded nervous system development and brain injury[11-14], ischemic or hemorrhagic cerebral stroke[15-17], pulmonary arterial hypertension or Eisenmenger syndrome[18-20], infective endocarditis[21-23], myocardial dysfunction or heart failure[24-28], ventricular or supraventricular arrhythmias[29-31],

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and cardiac demise[32-34]. Actually, CHD remains the most common etiology of infant death resulted from birth defects, with approximately 24% of neonates who died of a birth defect having CHD[2]. Although tremendous advance in cardiac surgery and catheter-based intervention, as well as perioperative intensive care, has dramatically improved survival, allowing more than 90% of CHD neonates to survive into adulthood, it leads to a growing body of adult population with CHD, and adults with CHD have outnumbered children with CHD[2,35]. Moreover, the late complications and even sudden cardiac death significantly increase in adult survivors living with CHD[36-38]. Hence, CHD has conferred a substantially increased socioeconomic burden[39-41]. Despite important clinical significance, the molecular etiologies of CHD remain largely obscure.

In vertebrates, the heart is the first functional organ that develops during embryogenesis, and cardiac morphogenesis is a complex biological process, which is finely controlled by a modulatory network, encompassing transcription factors, signaling molecules, and epigenetic modifiers[42]. Previous researches[42-46] have demonstrated that both environmental and genetic pathogenic factors may interrupt this biological process, giving rise to CHD. The nongenetic environmental risk factors for CHD include maternal conditions (such as viral infection, metabolic disorder, and lack of nutrition) and exposures to toxicants, therapeutic chemicals, and ionizing radiation during the first trimester of pregnancy[45,46]. However, accumulating investigations highlight the strong genetic basis for CHD, and in addition to chromosomal deletions and duplications, an increasing number of variations in over 100 genes, including those coding for cardiac transcription factors, cellular signaling molecules, and myocardial structural proteins, have been found to cause CHD in humans[42-44,47-69]. Among these well-established CHD-causing genes, most encode cardiac core transcription factors, encompassing GATA4, TBX20, GATA6, NKX2-5, GATA5, HAND1, and HAND2[70]. Nevertheless, the genetic determinants underpinning CHD in the vast majority of cases remain unknown. The current investigation was sought to identify a novel gene responsible for CHD and reveal the underlying mechanism by which the genetic variation contributes to CHD.

Subjects and Methods

Study Subjects

In this investigation, a three-generation Chinese family with CHD transmitted as an autosomal dominant trait was enrolled. Additionally, 236 index patients suffering from CHD were also included. The control individuals comprised 312 unrelated individuals with no heart disease. The CHD patients were matched with the healthy control subjects for ethnicity, sex, and age. Each study participant underwent a comprehensive clinical evaluation, including a thorough review of familial, personal and medical histories, a detailed physical examination, echocardiogram, electrocardiogram, and routine biochemical tests. Diagnosis of CHD was made by echocardiography, cardiac catheterization and/or cardiac surgery. Patients with known chromosomal abnormalities or syndromic CHD were ruled out from the current investigation. The investigation was carried out in conformity with the ethical principles outlined in the Declaration of Helsinki. The protocol used in this investigation was reviewed and approved by the Medical Ethics Committee of Tongji Hospital, Tongji University School of Medicine, Shanghai, China [Ethical Approval Number: LL(H)-09-07]. Prior to the commencement of the investigation, written informed consent was given by the study participants or their legal guardians. Subsequently, a peripheral venous blood sample (about 2 mL) was collected from each study subject. Genomic DNA was extracted from venous blood leucocytes with the FlexiGene DNA Kit (Qiagen, Hilden, Germany), then, stored at −80°C.

Genetic Analyses

For each sample, a whole exome library was constructed with 3 μg of genomic DNA by random fragmentation using an ultrasonicator (Covaris, Woburn, MA, USA), and captured using the SureSelect^{XT} Human All Exon V6 Kit (Agilent Technologies, Santa Clara, CA, USA), following the manufacturer's instructions. Enriched exome libraries were sequenced with the HiSeq Sequencing Kit (Illumina, San Diego, CA, USA) on an Illumina HiSeq 2000 Genome Analyzer (Illumina) according to the manufacturer's protocol. Exome sequences were mapped to the human reference genome sequence (hg19, GRCh37) using the BWA software. The resulting SAM files were converted to the BAM files using SAMtools, and duplicates were removed with the Picard software. The GATK software package was applied to base quality score recalibration, local realignment, and variant calls. The called variants that passed the pedigree analysis were annotated by virtue of the software ANNOVAR. The candidate variants identified by whole exome sequencing (WES)

Table I. Primers for amplification of the coding exons and splicing donors/acceptors of the KLF13 gene.

Coding exon	Forward primer (5′→3′)	Backward primer (5′→3′)	Amplicon (bp)
1-a	CCATGCGCTCACTCTTCGGT	CCTTTGTCTGAGGCCGGGCT	670
1-b	CGGACCTCAACCAGCAAGCG	CTCCGAGAGCCAAGACCCGC	569
2	GCATGTGGGAGGGGTGTTGA	TCGTGAAACGTGTCCATCCCT	675

and bioinformatics analyses of the CHD family were further analyzed by Sanger sequencing. For a confirmed genetic variant, the coding exons and flanking introns of the variant-carrying gene were amplified from the genomic DNA samples of 236 index patients and 312 unrelated control individuals, on a Thermocycler (Applied Biosystems, Foster, CA, USA) by Polymerase Chain Reaction (PCR) using the HotStar Taq DNA Polymerase (Qiagen, Shanghai, China) and the primers shown in Table I. The amplicons were sequenced under an ABI 3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's manual. For a validated genetic variation, the Single Nucleotide Polymorphism database (https://www.ncbi.nlm.nih.gov/SNP), the 1000 Genomes Project database (http://www.1000genomes.org), and the Genome Aggregation Database (https://gnomad.broadinstitute.org) were retrieved to check its novelty.

Gene Expression Plasmids and Site-Directed Mutagenesis

Isolation of total RNAs from human heart samples and preparation of cDNAs were described elsewhere[71,72]. The wild-type cDNAs of the human *KLF13* gene (accession no. NM_015995.3) were amplified by PCR using the pfuUltra high-fidelity DNA polymerase (Stratagene, Santa Clara, CA, USA) and a specific pair of primers (forward primer: 5′-GGTGAATTCGCGGATGCGCGGCTGACGAC-3′; reverse primer: 5′-TGCTCTAGAGCGGCTGCTCATGGCTGTGG-3′). The produced cDNAs of *KLF13* were doubly cut by restriction enzymes EcoRI and XbaI (NEB, Ipswich, MA, USA), purified with the QIAquick Gel Extraction Kit (Qiagen, Shanghai, China), and then inserted at the EcoRI-XbaI sites into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA) to construct a eukaryotic expression plasmid KLF13-pcDNA3.1. The variation identified in CHD patients was introduced into the wild-type KLF13-pcDNA3.1 plasmid by site-directed mutagenesis utilizing the QuickChange II XL Site-Directed Mutagenesis Kit (Stratagene, San Diego, CA, USA) with a complementary pair of primers (forward primer: 5′-AGCCCGGCGTGGAGCTAGCCGGAGCCCGAGG-3′; reverse primer: 5′-CCTCGGGCTCCGGCTAGCTCCACGCCGGGCT-3′), and was verified by Sanger sequencing. The expression plasmids GATA4-pSSRa and GATA6-pcDNA3.1 as well as the reporter plasmid atrial natriuretic peptide-luciferase (ANP-luc), which expresses firefly luciferase, were described previously[73,74]. The reporter plasmid α actin-Luciferase (ACTC1-luc) expressing firefly luciferase was constructed as described previously[75].

Cell Culture, Transfection and Luciferase Assays

NIH 3T3 cells were cultivated in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA) and 1% penicillin/streptomycin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), in an incubator with an atmosphere of 5% CO_2 at 37°C. Cells were seeded into a 24-well plate at a density of 1×10^5 per cell 24 h before transfection. Various plasmids were transfected into cells with the FuGENE® HD Transfection Reagent (Promega, Madison, WI, USA) according to the manufacturer's protocol. Specifically, the cells were transfected with empty pcDNA3.1 (100 ng), or wild-type KLF13-pcDNA3.1 (100 ng), or Glu124*-mutant KLF13-pcDNA3.1 (100 ng), or wild-type KLF13-pcDNA3.1 (50 ng) plus empty pcDNA3.1 (50 ng), or wild-type KLF13-pcDNA3.1 (50 ng) plus Glu124*-mutant KLF13-pcDNA3.1 (50 ng), in combination with ANP-luc (800 ng) and pGL4.75 (20 ng). For analysis of the synergistic transactivation, the same amount (100 ng) of each plasmid (empty pcDNA3.1, wild-type KLF13-pcDNA3.1, Glu124*-mutant KLF13-pcDNA3.1, GATA4-pSSRa, GATA6-pcDNA3.1) was used alone or together, in the presence of ANP-luc (1000 ng) and pGL4.75 (20 ng). The plasmid pGL4.75 (Promega, Madison, WI, USA), which expresses Renilla Luciferase, was co-transfected as an internal control to minimize the intra-ex-

perimental and inter-experimental variances in transfection efficiency. Cells were harvested 36 h after transfection. The Luciferase activities were measured under the GloMax-96 Microplate Luminometer (Promega, Madison, WI, USA) by using the Dual-Glo Luciferase Assay System (Promega, Madison, WI, USA) according to the manufacturer's protocol. The promoter activity was reported as fold activation of Firefly Luciferase relative to Renilla Luciferase. All transfection experiments were done at least three times in triplicate.

Statistical Analysis

Data for promoter activity were expressed as mean ± standard deviations (SD) of the results from three experiments in triplicate. Differences in promoter activity between two groups were compared by using an unpaired Student's *t*-test. A two-sided *p*-value of <0.05 was considered to indicate a significant difference.

Results

Clinical Characteristics of the Study Participants

In this investigation, a family affected with CHD (Figure 1A) was recruited from the Chinese Han population in China. The Chinese family consisted of 18 living members spanning three generations, of whom five members (three males and two females; ages ranging from 1 to 34 years) had double-outlet right ventricle (DORV) and ventricular septal defect (VSD), based on their echocardiographic findings. In this three-generation pedigree (Figure 1A), CHD was transmitted in an autosomal-dominant fashion with complete penetrance. The proband, a one-year-old male, was referred to our hospital for cardiac surgery due to CHD. The proband's other affected relatives had also experienced successful surgical procedures for the correction of CHD. The clinical features of the family members with CHD are given in Table II. Additionally, 236 other index patients suffering from CHD (138 males, with an average age of 5 years) and 312 unrelated individuals with no CHD (182 males, with a mean age of 5 years) were also investigated. The CHD index inpatients were matched with the healthy control individuals for gender, ethnicity, and age. All these index cases had echocardiograph-documented CHD, while the control people had normal cardiac echocardiograms, with no evidence of heart diseases. Among the index patients, isolated CHD accounted for 53%; whilst complex CHD accounted for 47%. Atrial fibrillation and atrioventricular block occurred in 29/236 and 14/236 index patients, respectively. Surgical repair and catheter-based closure were achieved in 139/236 and 71/236 index patients, respectively. Besides, 37 of 236 probands had a positive family history of CHD, whereas none of the 312 control subjects had it. No known environmental risk factors predisposing to CHD were ascertained in the study participants. The baseline clinical features of the 236 index cases with CHD are summarized in Table III.

Identification of a CHD-Causative KLF13 Variation

WES was performed in four affected family members (II-1, II-8, III-1, and III-6) and two unaffected family members (II-2 and II-7) of the family (Figure 1A), generating an average of 22 Gb of sequence for each family member, with an average of 97% mapping to the human reference genome (hg19) and 74% mapping to the target sequences. A mean of 16,902 exonic variants (ranging from 15,261 to 18,205) per family member passed inheritance model filtering, of which 12 heterozygous nonsense, missense, and splicing site variants passed ANNOVAR filtering, shared by the four affected family members, and predicted to be deleterious, with a minor allele frequency <0.001. Further genetic analyses showed that only the variant chr15:31619785G>T (GRCh37: NC_000015.9), equal to chr15:31327582G>T (GRCh38: NC_000015.10) or NM_015995.3: c.370G>T; p.(Glu124*), in the *KLF13* gene, was verified by Sanger sequencing and co-segregated with CHD in the whole family. This genetic variant has been deposited in Leiden Open Variation Database version 3.0 (LOVD v.3.0), with an individual number of 00307305 (https://databases.lovd.nl/shared/individuals/00307305). The chromatograms illustrating the heterozygous *KLF13* variant, as well as its homozygous wild-type control sequence, are shown in Figure 1B. The schematic diagrams displaying the structural domains of the wild-type and mutant KLF13 proteins are shown in Figure 1C. The truncating variant was neither detected in 624 control chromosomes nor reported in the Single Nucleotide Polymorphism database, the 1000 Genomes Project database, or the Genome Aggregation Database, indicating a novel variant. Besides, Sanger sequencing analysis of 236 other probands with CHD revealed no causative *KLF13* variant. In addition, in this

Table II. Phenotypic characteristics and *KLF13* mutation status of the pedigree members with congenital heart defects.

Individual (Family 1)	Gender	Age (years)	Cardiac phenotype	KLF13 mutation (c.370G>T)
I-1	M	53*	VSD, DORV	NA
II-1	M	34	VSD, DORV	+/–
II-5	M	29	VSD, DORV	+/–
II-8	F	27	VSD, DORV	+/–
III-1	F	9	VSD, DORV	+/–
III-6	M	1	VSD, DORV	+/–

DORV = double-outlet right ventricle; F = female; M = male; NA = not available; VSD = ventricular septal defect; +/– = heterozygote. *Age at death.

study, a rare heterozygous *KLF13* variant was identified in a family with CHD, and shown to have a loss-of-function effect. The variant was neither observed in another cohort of 236 index cases with CHD nor found in 312 unrelated healthy individuals used as controls. Hence, neither the association of the variant between cases and controls nor the Hardy-Weinberg equilibrium was performed.

Functional Failure of the KLF13 Variant

As shown in Figure 2, the wild-type and Glu124*-mutant KLF13 expression plasmids (each 100 ng) transcriptionally activated the

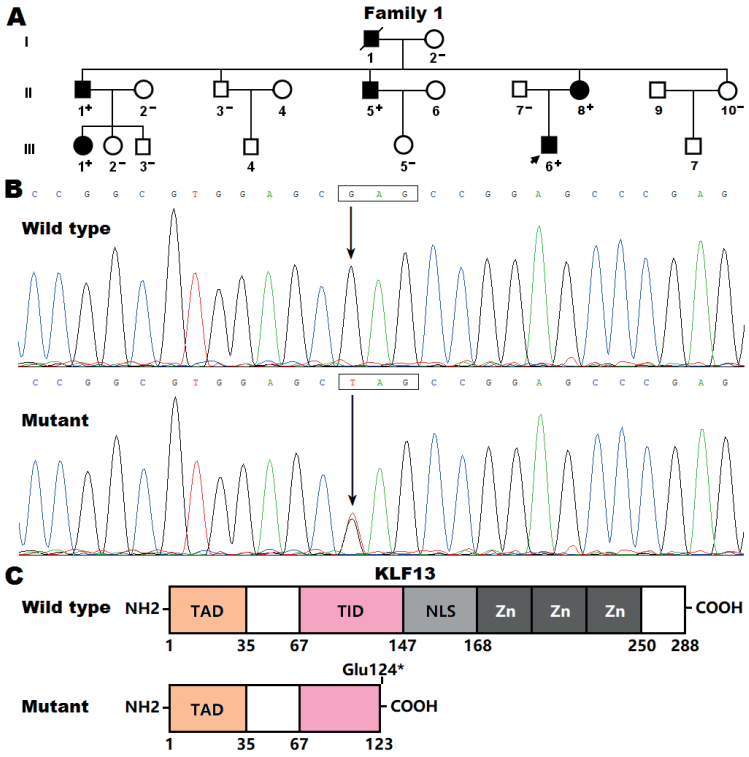

Figure 1. A novel *KLF13* variation responsible for familial congenital heart defects. Panel **(A)** exhibits the heterozygous *KLF13* variation from the proband (mutant) as well as its homozygous wild-type control from a healthy individual (wild type). A rectangle marks three adjacent nucleotides constituting a genetic codon, with an arrow pointing to the homozygous nucleotides of G/G (wild type) or the heterozygous nucleotides of T/G (mutant). Panel **(B)** displays the structural domains of the KLF13 proteins. NH2, amino-terminus; TAD, transcriptional activation domain; TID, transcriptional inhibitory domain; NLS, nuclear location signal; Zn, Zinc finger; COOH, carboxyl-terminus. Panel **(C)** shows the pedigree affected with congenital heart defects. Pedigree members are recognized by generations and numbers. Squares represent male family member; circles, female members; open symbols, unaffected members; closed symbols, affected members; a symbol with a slash, a deceased member; an arrow pointing to a closed square, an index patient; "+", a carrier of the heterogeneous *KLF13* variation; "–", a noncarrier.

Table III. Demographic and baseline clinical features of the study patients with congenital heart defects (n = 236).

Variable	n or mean ± SD	% or range
Demographics		
Male	138	58
Age (years)	5 ± 3	1-10
Positive family history of CHD	37	16
Distribution of distinct types of CHD		
Isolated CHD	125	53
VSD	35	15
ASD	30	13
PDA	22	9
DORV	11	5
TGA	8	3
PS	6	3
AS	5	2
PTA	3	1
IAA	3	1
CoA	2	1
Complex CHD	111	47
TOF	32	14
DORV + VSD	27	11
VSD + PDA	16	7
VSD + ASD	12	5
ASD + PDA	11	5
PTA + VSD	8	3
TOF + ASD	3	1
TGA + VSD	2	1
Incidence of arrhythmias		
Atrial fibrillation	29	12
Atrioventricular block	14	6
Treatment		
Surgical repair	139	59
Catheter-based closure	71	30
Follow-up	26	11

Data are presented as mean with standard deviations, number, or percentage.
AS = aortic stenosis; ASD = atrial septal defect; CHD = congenital heart defects; DORV = double-outlet right ventricle; IAA = interrupted aortic arch; PDA = patent ductus arteriosus; PS = pulmonary stenosis; PTA = persistent truncus arteriosus; SD = standard deviations; TGA = transposition of the great arteries; TOF = tetralogy of Fallot; VSD = ventricular septal defect.

ACTC1 promoter by ~10 fold and ~1 fold, respectively (wild type versus variant: $t = 9.59037$, $p = 0.00066$). When the wild-type and Glu124*-mutant KLF13 expression plasmids (each 50 ng) were used in combination, the induced transcriptional activity was ~5-fold (wild type plus empty plasmid versus wild type plus variant: $t = 4.31373$, $p = 0.01251$).

Disrupted Synergistic Activation Between KLF13 Variant and GATA4 as Well as GATA6

As shown in Figure 3, wild-type and Glu124*-mutant KLF13 activated the *ANP* promoter by ~12 fold and ~1 fold, respectively (wild type versus variant: $t = 11.0218$, $p = 0.00039$). In the presence of wild-type GATA4, wild-type and Glu124*-mutant KLF13 transcriptionally activated the *ANP* promoter by ~53 fold and ~7 fold, respectively (wild type versus variant: $t = 16.0378$, $p = 0.00009$); while in combination with wild-type GATA6, wild-type and Glu124*-mutant KLF13 transactivated the *ANP* promoter by ~46 fold and ~2 fold, respectively (wild type versus variant: $t = 17.6344$, $p = 0.00006$).

Discussion

In the present investigation, a three-generation Chinese family with CHD transmitted as an autosomal dominant trait was recruited. By WES and bioinformatics analyses of the family members, a novel heterozygous mutation, NM_015995.3: c.370G>T; p.(Glu124*), was identified in the *KLF13* gene. Genetic analysis of the whole pedigree

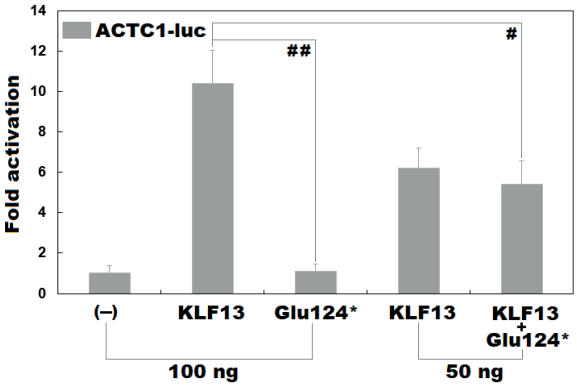

Figure 2. Functional loss of KLF13 resulted from the variation. Activation of α actin promoter-driven luciferase in cultivated NIH 3T3 cells by wild-type or Glu124*-mutant KLF13, alone or together, showed that the Glu124*-mutant KLF13 protein had no transcriptional activity. For each plasmid, cellular transfection experiments were conducted in triplicates and the resultant data are expressed as means together with standard deviations. Here ## and # indicate p <0.001 and p <0.013, respectively, when compared with wild-type KLF13.

showed that the mutation co-segregated with CHD, with complete penetrance. The mutation was neither observed in 312 unrelated healthy individuals nor found in such population genetics databases as the Single Nucleotide Polymorphism database, the 1000 Genomes Project database and the Genome Aggregation Database. Biological assays revealed that Glu124*-mutant KLF13 lost transcriptional activity on the promoters of *ACTC1* and *ANP*. Furthermore, the mutation abolished the synergistic transcriptional activation between KLF13 and GATA4, as well as GATA6, two other well-established CHD-causing genes. These findings strongly indicate that genetically defective *KLF13* contributes to CHD in the family.

In humans, *KLF13* maps on chromosome 15q13.3, coding for Kruppel-like factor 13 (KLF13), a protein with 288 amino acids. The KLF13 protein possesses four functionally important structural domains, including a transcriptional activation domain (TAD; amino acids 1-35), which is responsible for transactivation of target genes; a transcriptional inhibition domain (TID; amino acids 67-168), which is required for transcriptional inhibition of target genes; and two nuclear localization signal (NLS) domains, including NLS1 (amino acids 147-168) and NLS2 (amino acids 168-250) with three zinc-finger (Zn) motifs, of which NLS1 serves for nuclear localization, while NLS2 functions to bind target promoter DNAs and interact with other transcriptional cooperative partners[76]. Previous investigations[75,77,78] have corroborated that KLF13 as a cardiac core transcriptional factor plays a key role in cardiovascular development. During embryogenesis, KLF13 is highly expressed in the heart, where it transcriptionally mediates the expression of many target genes, including *ANP*, *BNP*, *ACTC1,* and *VEGFA*, singly or in synergy with GATA4, GATA6, and TBX5[75,77], and mutations in *KLF13* and its target genes *ACTC1* and *VEGF,* as well as its cooperative partners GATA4, GATA6, and TBX5 have been causally linked to CHD[78-86]. In the current research, the mutation identified in patients with familial CHD was predicted to create a truncated KLF13 protein lacking most functional domains, and functional studies demonstrated that the mutant KLF13 protein failed to transactivate its target genes. Furthermore, the mutation abrogated the synergistic transactivation between KLF13 and GATA4, as well as GATA6. These results indicate that *KLF13* haploinsufficiency is an alternative molecular mechanism of CHD in a subset of cases.

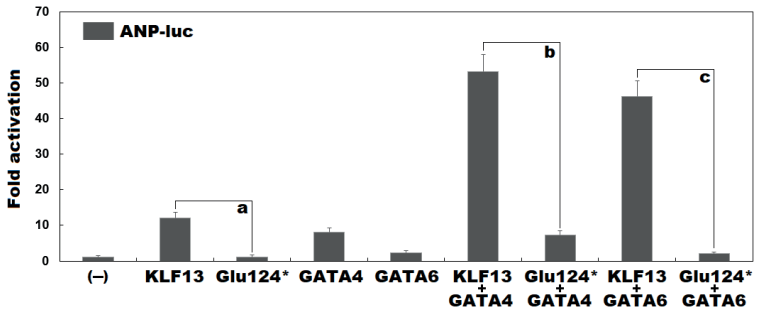

Figure 3. Abrogated synergistic activation between KLF13 variant and GATA4 as well as GATA6. The synergistic transcriptional activation of the promoter of atrial natriuretic peptide in cultivated NIH 3T3 cells by KLF13 and GATA4 as well as GATA5 was nullified by the Glu124* variation. For each plasmid, cellular transfection experiments were performed in triplicates, with means and standard deviations given. Here a, b and c indicate p <0.0005, p <0.0001 and p <0.0001, respectively, when compared with their wild-type counterparts.

It may be attributed to abnormal cardiovascular morphogenesis that *KLF13* loss-of-function mutation contributes to CHD. In xenopus, KLF13 is expressed predominantly in the heart during embryonic genesis, and knockdown of *Klf13* in developing embryos leads to atrial septal defects and hypotrabeculation, similar to those observed in humans or mice with hypomorphic *Gata4* alleles[75]. In mice, KLF13 is widely expressed at all embryo stages, and is highly expressed in the developing heart[87]. A spatiotemporal analysis of KLF13 expression in murine hearts revealed that the earliest sign of expression appeared at E9.5, subsequently with high levels of expression in the atrial myocardium, ventricular trabeculae, atrioventricular cushions and truncus arteriosus, which was then downregulated in the postnatal heart[75]. Mice with homozygous deletion of *Klf13* alleles had enlarged hearts and an increased susceptibility to cardiac vacuolar lesions and embryonic death[88]. Whereas mice with heterozygous knockout of *Klf13* showed no detectable cardiac defects, compound haploinsufficiency of *Klf13* and *Tbx5* significantly decreased the postnatal viability and increased the penetrance of *Tbx5*-dependent cardiac septal defects[75]. Taken collectively, these experimental results suggest that genetically compromised *KLF13* enhances the susceptibility to CHD in humans.

Notably, previous investigations have implicated *KLF13* mutations with sporadic CHD. Li et al[78] analyzed *KLF13* in 309 unrelated CHD patients by targeted sequencing and found two heterozygous variants, c.467G > A (Ser156Asn) and c.487C > T (Pro163Ser), in two sporadic CHD patients, respectively. The c.467G > A mutation carrier presented with VSD, tricuspid valve atresia, and atrial septal defect, while the c.487C > T mutation carrier presented with transposition of the great arteries. Functional deciphers showed that the variant Ser156Asn had increased protein expression and enhanced transactivation function, whereas the other variant Pro163Ser inhibited the transcriptional activity on downstream target genes[78]. Consistent with these observational results, heterozygous microdeletion and duplication of the human chromosomal region harboring *KLF13* (15q13.3) have been associated with a wide range of cardiac defects[89,90]. In the present study, a novel *KLF13* mutation, c.370G>T (Glu124*), was identified to contribute to familial CHD, including DORV and VSD, hence expanding the phenotypic spectrum linked to *KLF13* mutations. Notably, the current study for the first time implicates *KLF13* loss-of-function mutation with familial CHD, and firstly implicates *KLF13* loss-of-function mutation with DORV.

Conclusions

In summary, this investigation firstly links *KLF13* loss-of-function mutation to familial CHD, especially to DORV for the first time, providing novel insight into the molecular pathogenesis of CHD and implying potential implications for genetic counseling and improved personalized prophylaxis of CHD patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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