A novel *KLF13* mutation underlying congenital patent ductus arteriosus and ventricular septal defect, as well as bicuspid aortic valve

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Abstract. Recently, mutations in the Kruppel-like factor 13 (*KLF13*) gene encoding a Kruppel-like transcription factor have been reported to cause congenital heart disease (CHD). However, due to pronounced genetic heterogeneity, the mutational spectrum of *KLF13* in other cohorts of cases suffering from distinct types of CHD remain to be ascertained. In the present investigation, by Sanger sequencing of *KLF13* in 316 unrelated cases affected by different forms of CHD, a new mutation in heterozygous status, NM_015995.3: c.430G>T; p.(Glu144*), was detected in an index patient affected with patent ductus arteriosus (PDA) and ventricular septal defect (VSD), as well as bicuspid aortic valve (BAV), with a mutation frequency of ~0.32%. Genetic investigation of the available family members of the proband demonstrated that the truncating mutation co-segregated with CHD. The nonsense mutation was not observed in 400 unrelated volunteers without CHD who were enrolled as control subjects. Quantitative biological measurements with dual luciferase reporters revealed that Glu144*-mutant KLF13 did not transactivate the downstream genes vascular endothelial growth factor A and natriuretic peptide A. In addition, the mutation abrogated

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the synergistic transcriptional activation between KLF13 and T-box transcription factor 5, a well-established CHD-causing gene. In conclusion, the present study indicates that genetically defective *KLF13* contributes to familial PDA and VSD, as well as BAV, which expands the phenotypic spectrum linked to *KLF13*, and reveals a novel molecular pathogenesis of the disease, providing a new molecular target for the early prophylaxis and individualized treatment of CHD.

Introduction

Congenital heart disease (CHD), as a collective diagnosis for structural malformations of the heart and valves, as well as the endothoracic great blood vessels, occurring during embryonic development, represents the most common birth deformity in humans, with a prevalence of ~1% among live births worldwide (1,2). When minor cardiac structural anomalies are included, such as aneurysm of the atrial septum and bicuspid aortic valve (BAV), which is the most prevalent congenital cardiovascular anomaly with an incidence of 1-2% in the population, the overall prevalence of CHD may be as high as ~5% (2). Based on the occurrence of cardiac lesions in certain locations, CHD is clinically classified into >20 distinct subtypes, including ventricular septal defect (VSD), patent ductus arteriosus (PDA), transposition of the great arteries (TGA), double outlet of the right ventricle (DORV), tricuspid valve atresia (TVA), atrial septal defect (ASD), endocardial cushion defect, aortic stenosis, a right aortic arch, a single ventricle, tetralogy of Fallot, hypoplastic left heart and hypoplastic right heart (3-6). Irrespective of minor CHD that may resolve spontaneously (3), major CHD may contribute to diminished health-associated quality of life (7-9), decreased exercise performance (10-14), delayed neurodevelopment and brain injury (15-18), ischemic or hemorrhagic cerebral stroke (19-21), pulmonary arterial hypertension or Eisenmenger syndrome (22-24), viral pneumonia (25-27), infective endocarditis (28-30), acute myocardial infarction (31,32), chronic congestive heart failure (33-35), ventricular or supraventricular

arrhythmia (36-38) and death (39-42). Notably, CHD remains the most frequent etiology of newborn deaths caused by birth defects, with 21.8% of the neonates who succumb to various birth malformations having a cardiovascular abnormality (43). Although tremendous improvement in the outcomes of cardiac surgery and perioperative intensive care has been achieved, which allows >90% of CHD-infants to survive into adulthood and reach fertile age, this results in an increasing adult population with CHD, and now the rising number of adult patients with CHD accounts for more than two-thirds of the overall CHD population (44). Moreover, the rates of late surgical complication and cardiac comorbidity, and even mortality, markedly increase in adults affected with CHD (45-47). Despite the clinical importance, the etiologies underpinning CHD in a considerable proportion of cases remain obscure.

In vertebrates, the heart is the first functional organ that develops during embryonic morphogenesis, and cardiac organogenesis undergoes an extremely complex biological process, which is precisely mediated by a sophisticated regulatory network, involving transcription factors, cardiac structural proteins, signaling transducers, epigenetic modifiers and microRNAs (48). Previous studies have demonstrated that both environmental risk factors and genetic defects may interfere with this finely controlled developmental process, giving rise to CHD (1,2,48-54). The well-recognized non-inheritable pathogenic factors for CHD include maternal viral infection, nutritional deficiency, obesity, diabetes and decreased physical activity, as well as exposure to toxic chemicals, therapeutic drugs and ionizing radiation during early pregnancy (48-51). However, accumulating evidence highlights the strong genetic basis underpinning CHD (1,2,52-54). Significant familial aggregation of CHD has been reported, with the risk of CHD recurrence in the first-degree offspring of an affected parent being between 3 and 19% depending on the distinct types of lesion (55). In addition to chromosomal alterations encompassing aneuploidies, microdeletions and microduplications, pathogenetic variations in >100 genes amply expressed in the developing heart, encompassing those encoding sarcomeric proteins, transcription factors, chromatin modifies and signal-transducing molecules, have been determined to contribute to CHD (1,2,52,53,56-83). Of these reported CHD-causative genes, the majority code for cardiac core transcription factors, such as T-box transcription factor (TBX)1, TBX20, TBX5, NK2 homeobox 5, GATA binding protein (GATA)6, GATA4, GATA5, heart and neural crest derivatives expressed (HAND)1 and HAND2 (84). However, the genetic components underpinning CHD in most cases are still unknown.

Notably, mutations in the Kruppel-like factor 13 (*KLF13*) gene, which codes for a Kruppel-like transcription factor crucial for proper cardiovascular morphogenesis, have recently been discovered to cause distinct types of CHD (66,67). Li *et al* (66) performed targeted sequencing analyses of the entire coding region of *KLF13* in a cohort of 309 index patients suffering from CHD, and found two heterozygous *KLF13* variants in 2 out of 309 CHD patients, including NM_015995.3: c.467G>A; p.(Ser156Asn) in one patient affected with TGA and NM_015995.3: c.487C>T; p.(Pro163Ser) in another patient with TVA, VSD and ASD. These two missense mutations were absent from 200 population-matched healthy controls.

Biological assays elucidated that Ser156Asn-mutant KLF13 had enhanced transcriptional activation on the downstream target gene brain natriuretic peptide, alone or in synergy with TBX5, and a significantly enhanced ability to bind physically to TBX5, whereas the Pro163Ser variant showed a loss-of-function effect (66). Wang *et al* (67) performed whole-exome sequencing analyses in a family with a high incidence of CHD (DORV and VSD), and identified a new *KLF13* variant, NM_015995.3: c.370G>T; p.(Glu124*). The nonsense heterozygous mutation was absent from 312 control individuals without CHD. Functional investigation unveiled that the Glu124* variation exerted a loss-of-function impact on its two target genes, actin α cardiac muscle 1 (*ACTC1*) and atrial natriuretic peptide, singly or synergistically with GATA4, as well as GATA6 (67). These investigations underscore the substantial genetic heterogeneity of CHD comprising a wide spectrum of cardiovascular structural malformations, which encourages exploration of the spectrum and prevalence of *KLF13* variations in different cohorts of cases inflicted with various types of CHD. The aim of the current study was to analyze the spectrum and prevalence of *KLF13* variations in another cohort of cases with various types of CHD.

Materials and methods

Recruitment and clinical evaluation of study participants. The present study participants comprised a new cohort of 316 index patients affected with different forms of CHD enrolled from the Chinese Han population between March 2018 and November 2020 at Tongji Hospital and East Hospital, Tongji University (Shanghai, China). Clinical diagnosis and classification of various types of CHD were made as described previously (3,67). The relatives of the probands were also recruited when available. Patients with known syndromic CHD or chromosomal anomaly were ruled out from this research. Patients were diagnosed with syndromic CHD if they manifested a distinct facial gestalt or had at least one reported extra-cardiac malformation (85). A total of 400 unrelated volunteers without CHD were enrolled as control individuals from the same geographic area, who were exactly matched with the cases for sex and ethnicity, as well as age. All research participants underwent a comprehensive clinical assessment, as described previously (67-69). This research was fulfilled in compliance with the tenets of the Declaration of Helsinki. The protocol applied to the current investigation was approved by the Medical Ethics Committee of Tongji Hospital, Tongji University School of Medicine [Shanghai, China; approval no. LL(H)-09-07]. Written informed consent was provided by the research participants or their parents prior to commencement of sample collection.

Genetic analysis of KLF13. A whole blood specimen was collected from each study participant in an EDTA-coated tube and stored in a refrigerator at -80˚C. Genomic DNA was purified from blood leucocytes by utilizing DNA extraction reagent (Promega Corporation). The entire coding region, as well as splicing boundaries, of the *KLF13* gene (NC_000015.10) were amplified via polymerase chain reaction (PCR) using a DNA polymerase kit (Qiagen GmbH) and the *KLF13*-specific oligonucleotide primers, as described previously (67): Forward

5'-CCATGCGCTCACTCTTCGGT-3' and reverse, 5'-CCTTTG TCTGAGGCCGGGCT-3' for the first party of coding exon 1 (product size, 670 bp); forward, 5'-CGGACCTCAACCAGC AAGCG-3' and reverse, 5'-CTCCGAGAGCCAAGACCCGC-3' for the second party of coding exon 1 (product size, 569 bp); and forward, 5'-GCATGTGGGAGGGGTGTTGA-3' and reverse, 5'-TCGTGAAACGTGTCCATCCCT-3' for coding exon 2 (product size, 675 bp). Each mixture used for PCR was prepared in a 0.2-ml PCR tube with a final volume of $25\,\mu l$, containing 1X Buffer (Qiagen GmbH), 1X Q solution, a component of the HotStar Taq DNA Polymerase kit facilitating amplification of templates with a high-degree secondary structure or with a rich GC content by modifying the melting behavior of DNA (Qiagen GmbH), 0.2 mM dNTPs (Qiagen GmbH), 0.5 μM forward primer, 0.5 μM reverse primer, 0.02 U/μl HotStar Taq DNA Polymerase (Qiagen GmbH) and 0.1 μg genomic DNA. PCR was fulfilled on a 96-well thermocycler (Bio-Rad Laboratories, Inc.). The thermocycling conditions set for the PCR were as follows: Initial denaturation at 95°C for 15 min, followed by 36 cycles of denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec and extension at 72°C for 1 min, with a final extension at 72°C for 7 min. PCR products were resolved by 1.5% agarose gel electrophoresis and visualized after ethidium bromide staining of gels. PCR-sequencing of extracted amplicons was conducted as described previously (69). For a validated *KLF13* variation, the Human Gene Mutation Database (HGMD; http://www.hgmd. cf.ac.uk/ac/index.php), Single Nucleotide Polymorphism (SNP) datbase (https://www.ncbi.nlm.nih.gov/snp) and the Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org) were retrieved to verify its novelty.

Construction of gene expression plasmid and site-directed mutagenesis. The wild-type KLF13-pcDNA3.1 plasmid (Invitrogen; Thermo Fisher Scientific, Inc.) was constructed as described previously (67). The mutation discovered in the current study was introduced into wild-type KLF13-pcDNA3.1 by site-targeted mutagenesis with a site-targeted mutagenesis kit (Stratagene; Agilent Technologies Inc.) with the following primers: Forward, 5'-CCCGCGGGGAGCGGCTAGCCCGGC CTCAGAC-3' and reverse, 5'-GTCTGAGGCCGGGCTAGC CGCTCCCCGCGGG-3'. The mutant-type KLF13-pcDNA3.1 was selected by DpnI (Takara Biotechnology Co., Ltd.) and was confirmed by sequencing analysis. The TBX5-pcDNA3.1 plasmid (Invitrogen; Thermo Fisher Scientific, Inc.) and the reporter plasmid of human natriuretic peptide precursor A-luciferase (NPPA-luc), which expresses firefly luciferase, have been described previously (68). The reporter plasmid of human vascular endothelial growth factor A (VEGFA)-luc, which expresses firefly luciferase, was generated as previously described (86).

Cell culture, expression plasmid transfection and reporter gene assay. NIH3T3 cells (Cell Bank of Type Culture Collection of the Chinese Academy of Sciences) were seeded onto a 24-well plate, and maintained in DMEM (Merck KGaA) containing 10% fetal bovine serum and 1% penicillin/streptomycin (both Thermo Fisher Scientific, Inc.), in an atmosphere of 5% CO_2 at 37°C. NIH3T3 cells were transfected 24 h after plating with various expression plasmids, including empty pcDNA3.1, wild-type KLF13-pcDNA3.1 (KLF13),

Glu144*-mutant KLF13-pcDNA3.1 (Glu144*), wild-type TBX5-pcDNA3.1 (TBX5), NPPA-luc and VEGFA-luc, utilizing the Lipofectamine® 3000 Transfection Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) as described previously (67). The internal control plasmid pGL4.75 (Promega Corporation), which expresses *Renilla* luciferase, was co-transfected to balance transfection efficiency. The empty pcDNA3.1 plasmid (Invitrogen; Thermo Fisher Scientific, Inc.) was used as a negative control. Cells were collected at 48 h post-transfection, and lysed in 0.2 ml Reporter Lysis Buffer (Promega Corporation). The cellular lysates were used to measure the luciferase activities of firefly and *Renilla* on a luminometer (Promega Corporation), using a dual-luciferase assay kit (Promega Corporation). The activity of the target gene promoter was expressed as fold activation of firefly luciferase to *Renilla* luciferase. For each expression plasmid, three transfection experiments were performed in triplicate.

Statistical analysis. Data for promoter activity are presented as the mean ± standard deviation of the original results from three transfection experiments. Differences in promoter activities between two groups were compared with unpaired Student's t-test. When comparisons among multiple groups were made, one-way ANOVA with a Tukey-Kramer HSD post-hoc test was used. A two-sided P-value of <0.05 was used to indicate a statistically significant difference. The statistical software used for the analysis was SPSS version 17.0 for Windows (SPSS, Inc.).

Results

Clinical characteristic data of the studied patients. In this investigation, a cohort of 316 unrelated index patients suffering from various types of CHD (168 male cases and 148 female cases, with ages ranging from 1-49 years and a mean age of 21±9 years) was clinically investigated in contrast to a total of 400 unrelated individuals without CHD (212 male individuals and 188 female individuals, with ages ranging from 1-49 years and a mean age of 21±8 years). All the included patients with CHD had echocardiographic evidence, whereas the echocardiograms of the enrolled control subjects were normal, without evidence of cardiovascular structural abnormalities. Among the 316 unrelated index patients with CHD, 58 index patients reported a positive family history of CHD, while all 400 control subjects lacked a family history of CHD. No research participants had known environmental risk factors predisposing them to CHD, including maternal viral infection, nutritional deficiency, obesity, diabetes or exposure to toxic chemicals, therapeutic drugs and ionizing radiation during early pregnancy. Most of the patients underwent cardiac catheterization or surgery. The clinical features of the 316 index cases with CHD are summarized in Table I.

Detection of a new pathogenic KLF13 mutation. Via direct sequencing analysis of the entire coding region and splicing donors/acceptors of *KLF13* in 316 index patients with diverse forms of CHD, a heterozygous non-synonymous mutation, NM_015995.3: c.430G>T; p.(Glu144*), was detected in one index patient inflicted with CHD, comprising PDA and VSD, as well as BAV; this mutation therefore has a prevalence of ~0.32%

Table I. Demographic and clinical characteristics of the 316 patients affected with various forms of congenital heart disease.

Parameters	n or mean
Sex, n (%)	
Male	168 (53)
Female	148 (47)
Mean age (range), years	21±9 (1-49)
Distribution of distinct forms of CHD, n (%)	
VSD	73 (23)
ASD	57 (18)
PDA	38 (12)
TOF	32 (10)
DORV	16 (5)
VSD + PDA	28 (9)
VSD + ASD	22 (7)
DORV + VSD	16 (5)
TGA + VSD	16 (5)
ASD + PDA	9 (3)
TOF + ASD	6 (2)
PTA + VSD	3 (1)
Arrhythmias, n (%)	
AVB	16 (5)
FVPB	9 (3)
AF	7 (2)
PVT	3 (1)
Medical history, n (%)	
Cardiovascular surgery for CHD	177 (56)
Catheter-based treatment for CHD	101 (32)
Follow-up	38 (12)

CHD, congenital heart disease; VSD, ventricular septal defect; ASD, atrial septal defect; PDA, patent ductus arteriosus; DORV, double outlet of the right ventricle; TOF, tetralogy of Fallot; TGA, transposition of the great arteries; PTA, persistent truncus arteriosus; AVB, atrioventricular block; FVPB, frequent ventricular premature beat; AF, atrial fibrillation; PVT, paroxysmal ventricular tachycardia.

(1/316) in the study CHD population. The mutation carrier possessed a CHD-positive family history, and genetic assay of the available family members unveiled that the nonsense mutation was in co-segregation with autosomal-dominant CHD in the whole family, with complete penetrance for PDA, as well as BAV, and incomplete penetrance for VSD. The nonsense mutation was neither detected in 400 control subjects nor found in the HGMD, SNP and gnomAD databases, indicating its novelty. The sequence electropherogram traces illustrating the *KLF13* mutation in the heterozygous status as well as its wild-type sequence in the homozygous status are presented in Fig. 1A. The schemas exhibiting the functional domains of both wild-type KLF13 and mutant KLF13 are provided in Fig. 1B. The pedigree structure of the studied family inflicted with CHD is displayed in Fig. 1C. The clinical characteristic information of the CHD-affected family members is shown in Table II.

Transcriptional activation function of KLF13 is disabled by the mutation. As indicated in Fig. 2, wild-type KLF13 and Glu144*-mutant KLF13 (each 100 ng of expression plasmid) transactivated the *NPPA* promoter by ~16-fold and ~1-fold, respectively (wild-type KLF13 vs. Glu144*-mutant KLF13: t=9.99854, P=0.00056). When half the amount of wild-type KLF13 and Glu144*-mutant KLF13 (each 50 ng of expression plasmid) was utilized in combination to model the pathological state of mutation carriers (heterozygosity), the induced transcriptional activity was ~8-fold (wild-type KLF13 vs. wild-type KLF13 + Glu144*-mutant KLF13: t=5.31149, P=0.00604).

Synergistic transcriptional activation between KLF13 and TBX5 is nullified by the mutation. As indicated in Fig. 3, wild-type KLF13 and Glu144*-mutant KLF13 (each 100 ng of expression plasmid) transactivated the *VEGFA* promoter by ~9-fold and ~1-fold, respectively (wild-type KLF13 vs. Glu144*-mutant KLF13: t=9.90028, P=0.00058). In the presence of wild-type TBX5 (100 ng of expression plasmid), wild-type KLF13 and Glu144*-mutant KLF13 (each 100 ng of expression plasmid) transactivated the *VEGFA* promoter by ~36-fold and ~4 fold, respectively (wild-type KLF13 + wild-type TBX5 vs. Glu144*-mutant KLF13 + wild-type TBX5: t=16.2934, P=0.00008).

Discussion

In the present study, a new KLF13 mutation, NM_015995.3: c.430G>T; p.(Glu144*), was identified in one family suffering from PDA, BAV and VSD. The nonsense heterozygous mutation, which co-segregated with CHD in the whole family, was neither observed in 800 control chromosomes nor found in the databases of HGMD, SNP and gnomAD. Functional investigations demonstrated that the Glu144*-mutant KLF13 failed to transcriptionally activate the promoters of *NPPA* and *VEGFA*. Additionally, the mutation abrogated the synergistic transcriptional activation between KLF13 and TBX5, a well-established CHD-causative gene (68,69,84). These findings support the fact that genetically defective *KLF13* confers an enhanced susceptibility to CHD, including PDA, BAV and VSD. Notably, the experiments performed in one NIH3T3 cell line only failed to control for cell-dependent effects, and it remains possible, in fact likely, that other cells may yield different results. Hence, it is very important that additional cells lines are used to examine the functional effect of Glu144*-mutant KLF13 to generalize the mechanism proposed on the basis of this work.

The *KLF13* gene, which encodes a transcription factor with 288 amino acids, is located on human chromosome 15q13.3. As one member of the KLF family, the KLF13 protein harbors four critical structural domains encompassing a transactivation domain, which functions to transactivate downstream target genes, a transcriptional inhibition domain, which serves to transcriptionally inhibit downstream target genes, a nuclear localization signals responsible for nuclear localization, and three zinc-fingers required for binding to target gene promoters and interaction with other transcriptionally cooperative partners (67,87). Previous investigations have substantiated the ample expression of KLF13 in the hearts
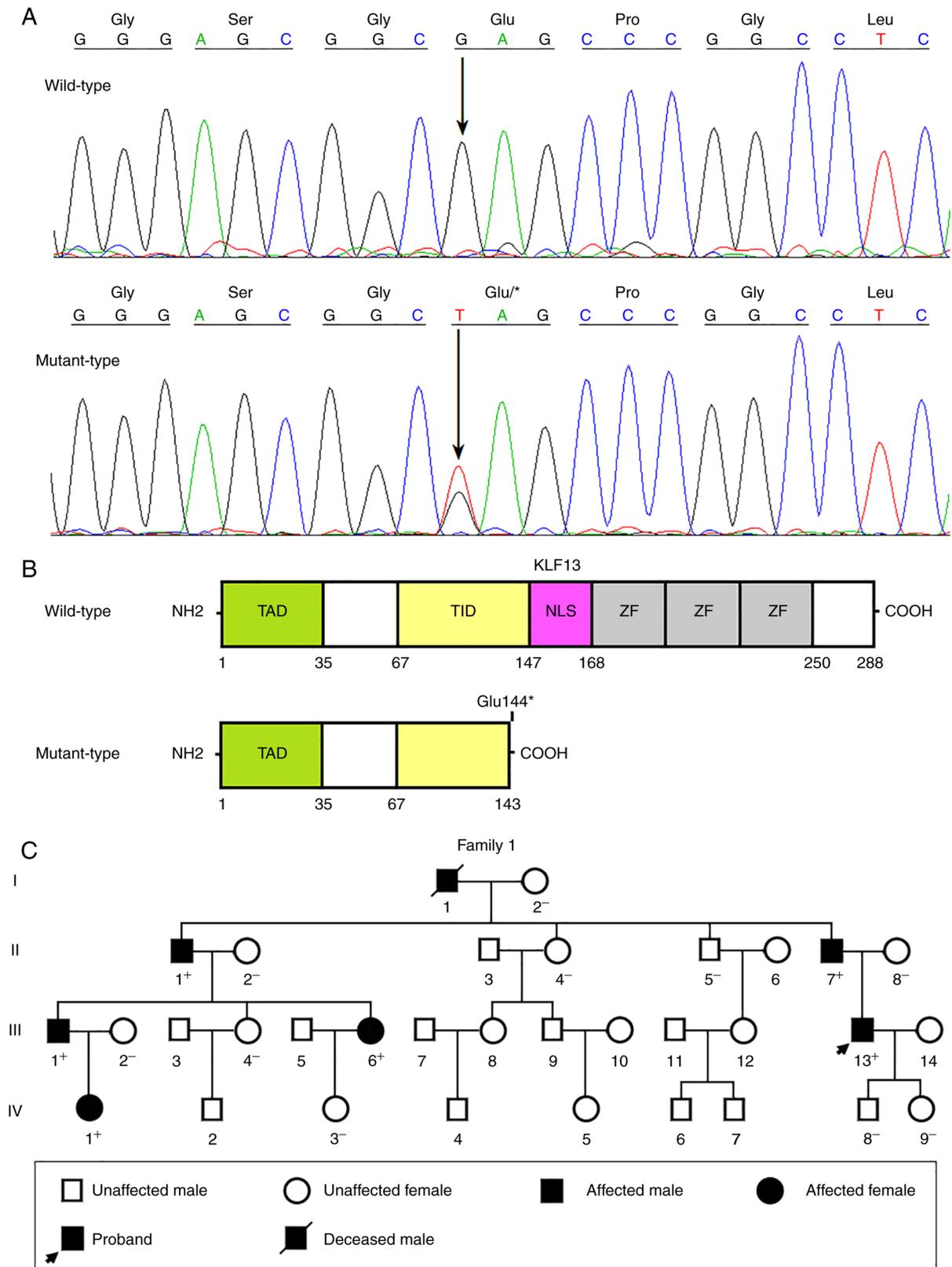
Figure 1. A new *KLF13* mutation contributing to congenital heart disease. (A) Sequence electropherograms illustrating the *KLF13* mutation in the heterozygous status (mutant) and its wild-type base in the homozygous status (wild-type). The arrow indicates the nucleotides where the mutation occurs. (B) Schematic representations exhibiting the structural domains of KLF13. (C) Pedigree structure of the family inflicted with congenital heart disease (+ represents a carrier of the *KLF13* mutation and-represents a non-carrier). TAD, transcriptional activation domain; TID, transcriptional inhibitory domain; NLS, nuclear location signal; Zn, zinc finger.

of both humans and vertebrates during embryonic development, and the pivotal effect of KLF13 on cardiovascular morphogenesis (66,67,86,88). Recent experimental studies have corroborated that KLF13 transactivates the expression

Table II. Phenotypic profile and KLF13 mutation status of the family members affected with congenital heart disease.

Individual	Sex	Age, years	Cardiac phenotype	KLF13 mutation (p.Glu144*)
I-1	M	75[a]	PDA, BAV, VSD	NA
II-1	M	61	PDA, BAV	+/-
II-7	M	53	PDA, BAV, VSD	+/-
III-1	M	36	PDA, BAV	+/-
III-6	F	30	PDA, BAV	+/-
III-13	M	28	PDA, BAV, VSD	+/-
IV-1	F	10	PDA, BAV	+/-

[a]Age at death. M, male; F, female; PDA, patent ductus arteriosus; BAV, bicuspid aortic valve; VSD, ventricular septal defect; NA, not available; +/-, heterozygosity; KLF13, Kruppel-like factor 13.
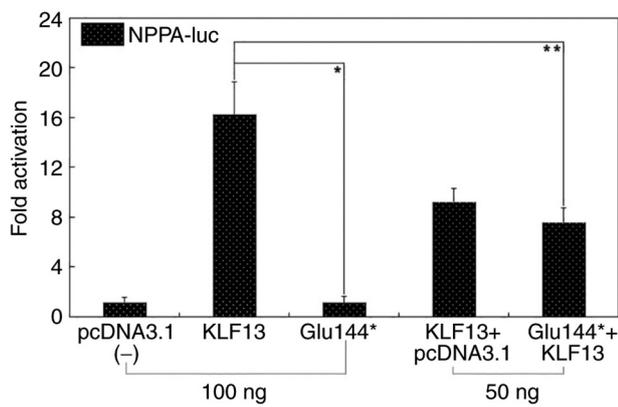

Figure 2. Diminished transcriptional activity of KLF13 caused by the mutation. In cultivated NIH3T3 cells expressing various recombinant plasmids, activation of NPPA-luc by wild-type or Glu144*-mutant KLF13, singly or together, revealed that Glu144*-mutant KLF13 failed to transactivate its downstream target gene *NPPA*. For each eukaryotic expression plasmid, cellular transient transfection experiments were repeated three times in triplicates. *P=0.00056 and **P=0.00604 compared with wild-type KLF13. NPPA-luc, human natriuretic peptide precursor A luciferase; KLF13, Kruppel-like factor 13.

Figure 3. Synergistic transcriptional activation between KLF13 and TBX5 disrupted by the mutation. In cultivated NIH3T3 cells, the synergistic transactivation of the promoter of VEGFA by KLF13 and TBX5 was abrogated by the Glu144* mutation. *P=0.00058 and **P=0.00008 compared with to respective wild-type counterparts. VEGFA, human vascular endothelial growth factor A luciferase; TBX5, T-box transcription factor 5; KLF13, Kruppel-like factor 13.

of several downstream genes, encompassing *NPPA*, *NPPB*, *VEGFA* and *ACTC1*, separately or synergistically with TBX5, GATA4 and GATA6 (86,88). Deleterious mutations in KLF13 and its downstream target genes VEGFA and ACTC1, as well as its transcriptionally cooperative partners TBX5, GATA4 and GATA6, have been discovered as genetic defects underpinning CHD in humans (66-69,84,89-91). In the current investigation, the mutation found in cases with familial CHD was anticipated to generate a truncated KLF13 protein losing most structural domains, and biochemical assays revealed that Glu144*-mutant KLF13 had no transactivation on its downstream target genes. In addition, the mutation disrupted the synergistic transcriptional activation between KLF13 and TBX5. These results strongly indicate *KLF13* haploinsufficiency as a molecular mechanism underpinning CHD in a subset of cases.

It has been validated in experimental animal models that *Klf13* deficiency contributes to aberrant cardiovascular morphogenesis. In *Xenopus*, KLF13 is abundantly expressed in the heart and vessels during embryogenesis, and knockdown of the *Klf13* alleles in *Xenopus* embryos leads to atrial
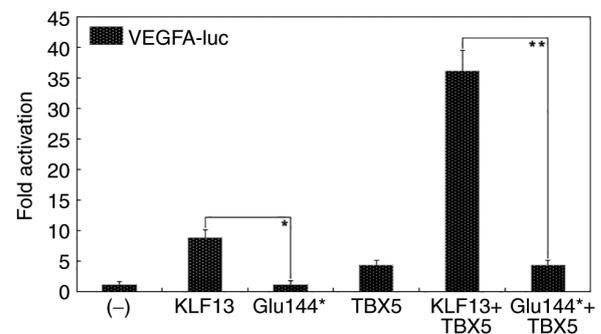
septal defects and myocardial trabecular hypoplasia, similar to those seen in mice or humans with hypomorphic alleles of *Gata4* (88). In mice, KLF13 is expressed highly in the heart at all stages of embryonic development, including during development of truncus arteriosus, ventricular trabeculae, atrial myocardium and atrioventricular cushions (88,92). When homozygous knockout of *Klf13* alleles was performed in mice, cardiac vacuolar lesions, heart enlargement and embryonic death occurred (93). In another study, although mice with heterozygous deletion of *Klf13* had no obvious cardiac structural abnormalities, compound haploinsufficiency of *Tbx5* and *Klf13* markedly reduced the postnatal viability and significantly increased the penetrance of cardiac septal aberrations caused by *Tbx5* haploinsufficiency (88). Altogether, these results from experimental animals indicate that genetically defective *KLF13* increases the vulnerability to CHD in humans, and suggest that the dosage of KLF13 must be finely controlled to avoid cardiovascular developmental malformations.

Notably, *KLF13* variations have been reported to cause distinct types of congenital cardiovascular deformities in humans, including TGA, TVA, DORV, VSD and ASD (66,67). In the present study, the affected family members who carried an identified *KLF13* mutation manifested PDA, BAV and VSD, therefore expanding the phenotypic spectrum ascribed to mutant *KLF13* and highlighting the genetic heterogeneity of CHD.

In conclusion, the present study causally links a new *KLF13* mutation to CHD, and to the best of our knowledge, for the first time to PDA and BAV, which reveals a novel molecular pathogenesis underlying CHD, conducive to precise prophylaxis and personalized treatment of the affected patients.

Acknowledgements

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JW and YQY designed the investigation, and made leading contributions to the writing of manuscript. PA, GFZ, CMZ, YJX, JW and YQY completed the clinical investigations. PA, GFZ, JW and YQY fulfilled the genetic and biochemical studies. JW and YQY confirm the authenticity of all the raw data. All authors have read and approved the final version of manuscript.

Ethics approval and consent to participate

This investigation was approved by the Medical Ethics Committee of Tongji Hospital, Tongji University School of Medicine [Shanghai, China; approval no. LL(H)-09-07]. Written informed consent was collected from the study individuals or their parents prior to investigation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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