

# A novel PITX2c loss-of-function mutation underlies lone atrial fibrillation

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**Abstract.** Atrial fibrillation (AF) is the most common form of sustained cardiac arrhythmia responsible for substantial morbidity and significantly increased mortality rates. A growing body of evidence documents the important role of genetic defects in the pathogenesis of AF. However, AF is a heterogeneous disease and the genetic determinants for AF in an overwhelming majority of patients remain unknown. In the present study, a cohort of 100 unrelated patients with lone AF and a total of 200 unrelated, ethnically matched healthy individuals used as controls, were recruited. The whole coding exons and splice junctions of the pituitary homeobox 2c (*PITX2c*) gene, which encodes a paired-like homeobox transcription factor required for normal cardiovascular morphogenesis, were sequenced in the 100 patients and 200 control subjects. The causative potential of the identified mutation of *PITX2c* was predicted by MutationTaster and PolyPhen-2. The functional characteristics of the *PITX2c* mutation were assayed using a dual-luciferase reporter assay system. Based on the results, a novel heterozygous *PITX2c* mutation (p.T97A) was identified in a patient with AF. The missense mutation was absent in the

400 reference chromosomes and was automatically predicted to be disease-causing. Multiple alignments of *PITX2c* protein sequences across species revealed that the altered amino acid was completely conserved evolutionarily. Functional analysis demonstrated that the mutant *PITX2c* protein was associated with significantly decreased transcriptional activity when compared with its wild-type counterpart. The findings of the present study firstly link the *PITX2c* loss-of-function mutation to lone AF, and provide novel insight into the molecular mechanisms underlying AF, suggesting the potential implications for the early prophylaxis and allele-specific therapy of this common type of arrhythmia.

## Introduction

Atrial fibrillation (AF), a supraventricular tachyarrhythmia characteristic of the rapid and chaotic electrical activity of the atria with consequent deterioration of atrial mechanical function, is the most prevalent type of sustained cardiac arrhythmia in humans, afflicting roughly one-third of all hospitalized patients with miscellaneous heart rhythm disturbances (1). The prevalence of AF is approximately 1% in the general population and increases drastically with advancing age, increasing from <1% in individuals aged <60 years up to ~10% in those >80 years of age (1). According to the report from the Framingham Heart Study, the lifetime risk of developing AF is approximately one in four among subjects older than 40 years (2). In the United States, the number of individuals affected by AF is currently estimated at 2.3 million and is projected to exceed 10 million by 2050 (3). This common cardiac dysrhythmia may lead to poor quality of life, decreased exercise tolerance, cognitive impairment, tachycardiomyopathy, thromboembolism, congestive heart failure and even death (1). The mortality rate of patients with AF is approximately twice that of age- and gender matched patients with normal heart rhythm, which persists even after the adjustment for pre-existing cardiovascular conditions (4). Patients with AF are at a 2- to 7-fold increased risk of ischemic stroke compared with those without AF and 15 to 20% of all strokes

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are attributed to AF (5). Notably, the risk of thromboembolic stroke ascribed to AF also increases steeply with increasing age, ranging from 1.5% for persons aged 50 to 59 years to 23.5% for persons aged 80 to 89 years (5). Hence, AF has imposed a heavy economic burden on patients and health care systems and the socioeconomic burden is anticipated to increase drastically in the future with life expectancies increasing (6). Despite the high prevalence and important clinical significance, the molecular mechanisms underlying AF remain poorly understood.

Traditionally, AF has been referred to as a complication of diverse cardiac and systemic disorders, including coronary heart disease, rheumatic heart disease, congenital heart disease, pulmonary heart disease, cardiomyopathy, cardiothoracic surgery, myocarditis, congestive heart failure, essential hypertension, diabetes mellitus and hyperthyroidism (1). However, in 12 to 30% of all AF patients and 20 to 45% of younger AF patients, AF occurs separately in the absence of underlying diseases or precipitating factors, a condition termed as lone or idiopathic AF, of which at least 15% of cases have a positive family history, thus defined as familial AF (1,7). A growing body of epidemiological research has demonstrated the familial aggregation of AF and the increased vulnerability to AF in the close relatives of patients with AF, strongly suggesting that genetic risk factors play a pivotal role in the pathogenesis of AF in a subset of patients (8-14). In previous studies, using genome-wide scanning with polymorphic microsatellite markers and linkage analysis, AF susceptibility loci were mapped on human chromosomes 10q22, 6q14-16, 11p15.5, 5p15, 10p11-q21 and 5p13, where AF-causative mutations in two genes, potassium voltage-gated channel, KQT-like subfamily, member 1 (*KCNQ1*) on chromosome 11p15.5 and nucleoporin 155 kDa (*NUP155*) on chromosome 5p13, were identified and functionally characterized (15-21). Direct analyses of candidate genes unveiled a high number of AF-related genes, including potassium voltage-gated channel, Isk-related family, member 2 (*KCNE2*), potassium voltage-gated channel, subfamily H (eag-related), member 2 (*KCNH2*), potassium inwardly-rectifying channel, subfamily J, member 2 (*KCNJ2*), potassium voltage-gated channel, shaker-related subfamily, member 5 (*KCNA5*), sodium channel, voltage-gated, type V, alpha subunit (*SCN5A*), sodium channel, voltage-gated, type IV, beta subunit (*SCN4B*), atrial natriuretic peptide (ANP), gap junction protein, alpha 5, 40 kDa (*GJA5*), GATA binding protein (GATA)4, GATA5, GATA6 and NK2 homeobox 5 (*NKX2-5*) (22-42). Nevertheless, AF is a genetically heterogeneous disease and the genetic basis underlying AF in an overwhelming majority of patients remains to be elucidated.

In a previous study, Gudbjartsson *et al* (43) performed a genome-wide association scan, followed by replication studies in three populations of European descent and a Chinese population and found a strong association between two sequence variants (rs2200733 and rs10033464) on chromosome 4q25 and AF. They demonstrated that ~35% of individuals of European descent had at least one of the variants and that the risk of AF increased by 1.72 and 1.39 per copy. The association with the stronger variant is replicated in the Chinese population, where it was carried by 75% of individuals, and the risk of AF was increased by 1.42 per copy. Subsequently, Käb *et al* (44) also confirmed the strong association of the

non-coding polymorphisms, rs2200733 and rs10033464, with AF in four independent cohorts of European descent. In two independently collected cardiac surgery cohorts, the two variants within the chromosome 4q25 region were independently associated with post-operative AF following coronary artery bypass graft surgery after adjusting for clinical covariates (45). Furthermore, the two sequence variants have been reported to increase the risk of the early and late recurrence of AF following catheter ablation (46) and have been shown to be genetic modifiers of rare ion channel mutations linked to familial AF (47). The gene closest to these sequence variants is pituitary homeobox 2 (*PITX2*), a member of the PITX family of transcription factors that play an essential role in embryonic morphogenesis, with *PITX2c* being the predominant isoform expressed in the embryonic and adult heart (48). Emerging evidence highlights the pivotal role of *PITX2c* in the embryonic development of the left atrium, the cardiac conduction system and pulmonary venous myocardium, a major source of ectopic activity involved in the initiation and maintenance of AF (49,50); the abnormal expression of *PITX2c* has also been implicated in the increased susceptibility to AF (51-53). These findings warrant the screening of *PITX2c* as a preferred candidate gene for lone AF.

## Materials and methods

**Study subjects.** A cohort of 100 unrelated patients with lone AF was enlisted from the Han Chinese population. A total of 200 ethnically matched, unrelated healthy individuals were enrolled as the controls. Peripheral venous blood samples were prepared and clinical data, including medical records, electrocardiogram and echocardiography reports were collected. The study subjects were clinically classified using a consistently applied set of definitions (7,34). Briefly, AF was diagnosed by a standard 12-lead electrocardiogram demonstrating no P waves and irregular R-R intervals irrespective of clinical symptoms. Lone AF was defined as AF occurring in individuals <60 years of age without other cardiac or systemic diseases by physical examination, electrocardiogram, transthoracic echocardiogram and extensive laboratory tests. Subjects were classified as 'healthy' if they were asymptomatic and had a normal electrocardiogram with no history of AF. Paroxysmal AF was defined as AF lasting >30 sec that terminated spontaneously. Persistent AF was defined as AF lasting >7 days and requiring either pharmacological therapy or electrical cardioversion for termination. AF that was refractory to cardioversion or that was allowed to continue was classified as permanent. The present study was performed in compliance with the ethical principles of the revised Declaration of Helsinki (48th WMA General Assembly, Somerset West, Republic of South Africa, 1996). The study protocol was reviewed and approved by the local institutional ethics committee and written informed consent was obtained from all participants prior to enrollment in this study.

**Genotyping for *PITX2c*.** Genomic DNA was extracted from the peripheral venous blood lymphocytes of all the participants using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA). The coding exons and intron-exon boundaries of *PITX2c* were sequenced in the 100 unrelated patients

Table I. Intronic primers used to amplify the coding exons and exon-intron boundaries of PITX2c.

Exon	Forward primer (5' to 3')	Reverse primer (5' to 3')	Amplicon size (bp)
1	CAG CTT GGC TTG AGA ACT CG	TGA CTT CCT TGG GGC GAG AG	442
2	CAG CTC TTC CAC GGC TTC TG	GCT GCC TTC CAC ATT CTC TC	387
3	AAT CTG CAC TGT GGC ATC TG	AGT CTT TCA AGG GCG GAG TT	677

PITX2c, pituitary homeobox 2c.

with lone AF. The 200 control individuals were subsequently genotyped for *PITX2c* due to the identified mutation in patients with AF. The referential genomic DNA sequence of *PITX2c* was derived from GenBank [accession no. NC\_000004; available at the National Center for Biotechnical Information (NCBI; <http://www.ncbi.nlm.nih.gov/>)]. With the aid of the on-line Primer 3 program (<http://frodo.wi.mit.edu>), the primers used to amplify the entire coding region and splice junctions of *PITX2c* by polymerase chain reaction (PCR) were designed as presented in Table I. PCR was carried out using HotStar Taq DNA Polymerase (Qiagen, Hilden, Germany) on a Veriti Thermal Cycler (Applied Biosystems, Foster City, CA, USA), with standard conditions and concentrations of reagents. Amplified products were analyzed on 1% agarose gels stained with ethidium bromide and purified using the QIAquick Gel Extraction kit (Qiagen). Both strands of each amplicon were sequenced with a BigDye<sup>®</sup> Terminator v3.1 Cycle Sequencing kit under an ABI PRISM 3130xl DNA Analyzer (both from Applied Biosystems). The sequencing primers were the same as those previously designed for specific region amplification. The DNA sequences were viewed using DNA Sequencing Analysis software v5.1 (Applied Biosystems). The variant was validated by re-sequencing an independent PCR-generated amplicon from the subject and met the quality control thresholds with a call rate >99%. Additionally, for an identified sequence variant, the Exome Variant Server (EVS; <http://evs.gs.washington.edu/EVS>) and the NCBI single nucleotide polymorphism (SNP; <http://www.ncbi.nlm.nih.gov/SNP>) online databases were queried to confirm its novelty.

**Multiple alignments of PITX2c protein sequences.** Multiple PITX2c protein sequences across various species were aligned using an online program MUSCLE, v3.6 (<http://www.ncbi.nlm.nih.gov/>).

**Prediction of the causative potential of a PITX2c sequence variation.** The disease-causing potential of a PITX2c sequence variation was predicted by the online program MutationTaster (<http://www.mutationtaster.org>), which automatically yielded a probability for the variation to be either a pathogenic mutation or a benign polymorphism. Of note, the P-value used in this study is the probability of the correct prediction rather than the probability of error as used in t-test statistics (i.e., a value close to 1 indicates a high accuracy of the prediction). In addition, another online program PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2>) was also used to evaluate the pathogenic likelihood of a mutation.

**Expression plasmids and site-directed mutagenesis.** The recombinant expression plasmid, PITX2c-pcDNA4, which was constructed by Strungaru *et al* (54), was a gift from Professor Georges Christé, Physiopathologie des Troubles du Rythme Cardiaque, Faculté de Pharmacie de Lyon, Université Lyon 1, Lyon, France. The atrial natriuretic factor (ANF)-luciferase reporter plasmid, which contains the 2600-bp 5'-flanking region of the ANF gene, namely ANF(-2600)-Luc, was kindly provided by Dr Ichiro Shiojima, from the Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Medicine, Chuo-ku, Chiba, Japan. The identified mutation was introduced into the wild-type PITX2c using the 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.

**Luciferase reporter gene assay.** Chinese hamster ovary (CHO) cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin. The CHO cells were cultured 24 h prior to transfection. The ANF(-2600)-Luc reporter construct and an internal control reporter plasmid pGL4.75 (hRluc/CMV; Promega) were used in transient transfection assays to explore the transactivational activity of the PITX2c mutant. CHO cells were transfected with 2 µg of wild-type PITX2c-pcDNA4 or mutant PITX2c-pcDNA4 or empty vector pcDNA4, 2.0 µg of ANF(-2600)-Luc reporter construct and 0.04 µg of pGL4.75 control reporter vector using Lipofectamine<sup>®</sup> 2000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA). For the co-transfection experiments, 1 µg of wild-type PITX2c-pcDNA4, 1 µg of mutant PITX2c-pcDNA4, 2.0 µg of ANF(-2600)-Luc and 0.04 µg of pGL4.75 were used. Transfected cells were incubated for 24 h, then lysed and assayed for reporter activities. Firefly luciferase and Renilla luciferase activities were measured using the Dual-Glo Luciferase Assay System (Promega). The activity of the ANF promoter was presented as the fold activation of Firefly luciferase relative to Renilla luciferase. At least three independent experiments were performed for wild-type or mutant PITX2c.

**Statistical analysis.** The continuous data are expressed as the means ± standard deviation. Continuous variables were tested for normality of distribution and the Student's unpaired t-test was used to compare numeric variables between two groups. The categorical variables were compared between two groups

Table II. Baseline clinical characteristics of the 100 unrelated patients with lone AF.

Parameter	No. or mean	% or range
Male	53	53
Age at initial diagnosis of AF (years)	45.8	20-60
Age at the time of present study (years)	52.1	21-67
Type of AF at presentation		
Paroxysmal AF	62	62
Persistent AF	26	26
Permanent AF	12	12
Positive family history of AF	5	5
History of cardioversion	82	82
Implanted cardiac pacemaker	2	2
Resting heart rate (beats/min)	78.6	56-164
Systolic blood pressure (mmHg)	132.7	90-138
Diastolic blood pressure (mmHg)	81.2	70-86
Body mass index (kg/m <sup>2</sup> )	22.4	20-24
Left atrial dimension (mm)	36	30-40
Left ventricular ejection fraction	0.6	0.5-0.7
Fasting blood glucose (mmol/l)	4.6	3.5-5.8
Total cholesterol (mmol/l)	4.5	3.4-6.2
Triglycerides (mmol/l)	1.5	0.9-1.7
Medications		
Amiodarone	78	78
Warfarin	65	65
Digoxin	12	12
β-blocker	10	10
Calcium channel blocker	6	6

AF, atrial fibrillation.

using Pearson's Chi-squared test or Fisher's exact test, where appropriate. A two-sided P-value <0.05 was considered to indicate a statistically significant difference.

## Results

**Clinical characteristics of the study population.** A cohort of 100 unrelated patients with lone AF was recruited, clinically evaluated and compared with 200 ethnically matched, unrelated healthy individuals. None of the participants had traditional risk factors for AF. There were no significant differences between the patient and control groups as regards baseline characteristics, including gender, body mass index, blood pressure, fasting blood glucose levels, serum lipid levels, left atrial dimension, left ventricular ejection fraction, heart rate at rest, as well as life style (data not shown). The

baseline clinical characteristics of the 100 patients with lone AF are summarized in Table II.

**PITX2c mutation.** A heterozygous missense mutation in PITX2c was identified in one of the 100 unrelated patients with lone AF. The total population prevalence of PITX2c mutation based on the patient cohort was 1%. Specifically, a substitution of guanine (G) for adenine (A) in the first nucleotide of codon 97 (c.289A>G), predicting the transition of threonine (T) into alanine (A) at amino acid position 97 (p.T97A), was identified in a male patient aged 46 years, who was initially diagnosed with AF at the age of 32. The mutation carrier had no apparent malformations in the eyes, teeth, umbilicus, or heart and had no positive family history of AF. No relatives from the mutation carrier were available for the genotyping of PITX2c. The sequence chromatograms showing the detected heterozygous PITX2c mutation of c.289A>G in contrast to its control sequence is shown in Fig. 1. A representative electrocardiogram of the mutation carrier showing AF is displayed in Fig. 2. A schematic diagram of PITX2c showing the structural domains and the location of the identified mutation is exhibited in Fig. 3 [modified from previous studies (55,56)]. This missense mutation was neither observed in the control population nor reported in the EVS and NCBI SNP databases.

**Alignment of multiple PITX2c protein sequences across species.** A cross-species alignment of PITX2c protein sequences revealed that the altered amino acid was completely conserved evolutionarily (Fig. 4).

**Disease-causing potential of the PITX2c variation.** The PITX2c sequence variation of c.289A>G was automatically predicted by MutationTaster to be a disease-causing mutation with the P-value of 1.0. No SNP in the altered region was found in the MutationTaster database. This PITX2c sequence variation was also predicted by PolyPhen-2 to be possibly damaging, with a score of 1.000 (sensitivity 0.00; specificity 1.00).

**Transcriptional activity of the PITX2c mutant.** The same amounts of wild-type PITX2c (2 μg) and T97A-mutant PITX2c (2 μg) activated the ANF promoter (~8- and 2-fold increase, respectively), when compared with the empty plasmid. When the same amount of wild-type PITX2c (1 μg) was transfected in combination with T97A-mutant PITX2c (1 μg), the induced activation of the ANF promoter was increased by ~4-fold, in comparison with the empty plasmid (Fig. 5).

## Discussion

In the present study, a novel heterozygous PITX2c mutation, p.T97A, was identified in a patient with lone AF, which was absent in the 400 reference chromosomes and was predicted to be pathogenic by MutationTaster and PolyPhen-2. A cross-species alignment of PITX2c protein sequences indicated that the altered amino acid was completely conserved evolutionarily. Functional analysis demonstrated that the mutant PITX2c protein was associated with a significantly reduced transcriptional activity. Therefore, it is likely that functionally compromised PITX2c predisposes to AF.

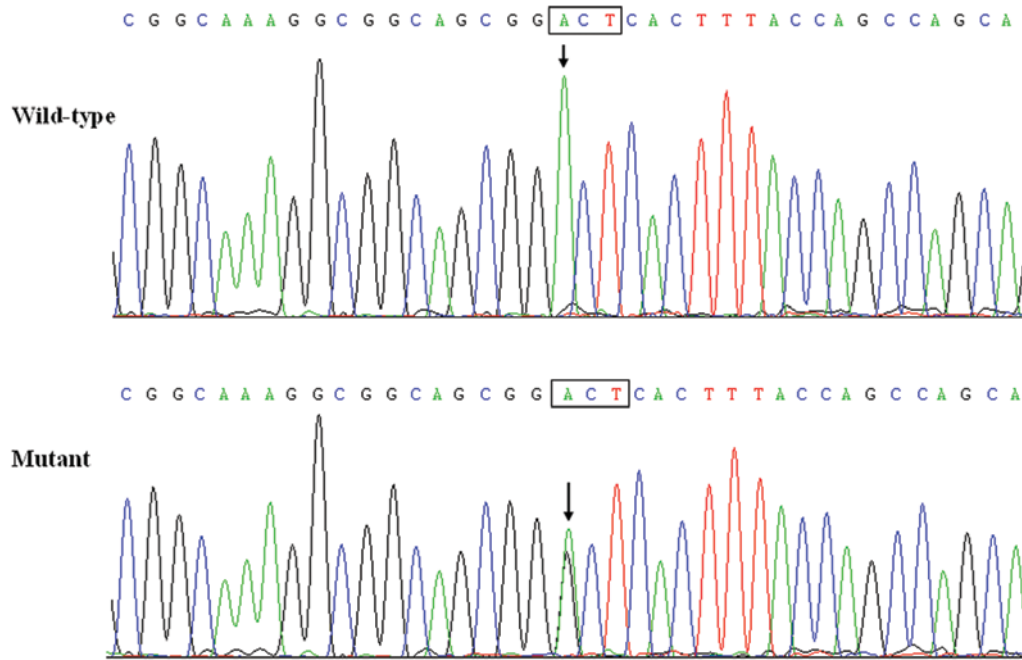


Figure 1. Sequence electropherograms showing the pituitary homeobox 2c (PITX2c) mutation in contrast with its corresponding control. The arrow indicates the heterozygous nucleotides of A/G (mutant) or the homozygous nucleotides of A/A (wild-type). The rectangle denotes the nucleotides comprising a codon of PITX2c.

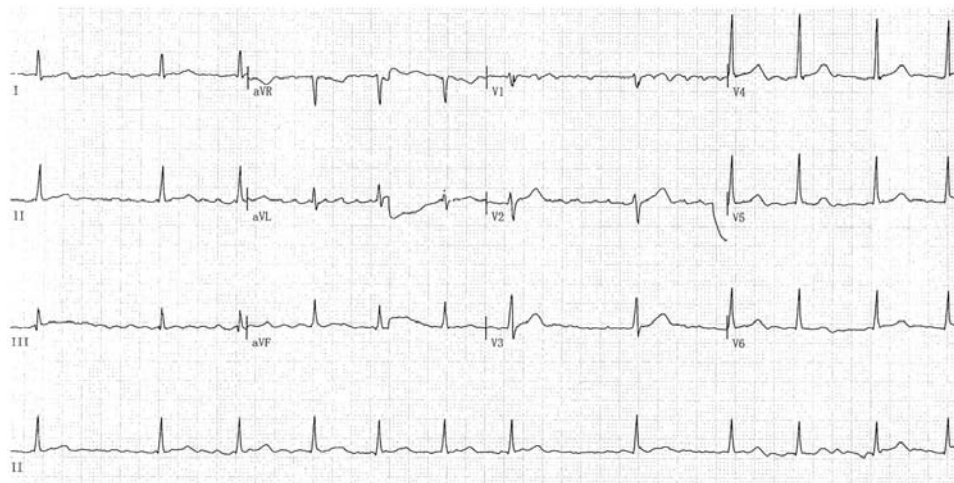


Figure 2. A standard 12-lead electrocardiogram from the mutation carrier with atrial fibrillation.

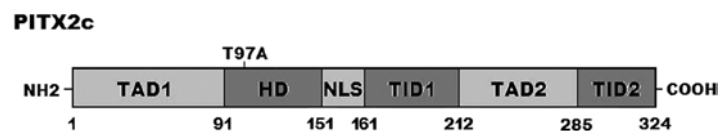


Figure 3. Schematic diagram of pituitary homeobox 2c (PITX2c) protein structure with the atrial fibrillation (AF)-related mutation indicated. The mutation identified in a patient with lone AF is shown above the structural domains. NH2, amino-terminus; TAD1, transcriptional activation domain 1 (amino acids 1-90); HD, homeodomain (amino acids 91-151); NLS, nuclear localization signal (amino acids 145-161); TID1, transcriptional inhibitory domain 1 (amino acids 162-212); TAD2, transcriptional activation domain 2 (amino acids 213-285); TID2, transcriptional inhibitory domain 2 (amino acids 286-324); COOH, carboxyl-terminus.

PITX2 is a member of the bicoid-like homeobox transcription factor family. Four different PITX2 transcripts, derived from alternative promoter usage and differential mRNA splicing, have been described. PITX2a, PITX2b and PITX2c

differ only in their amino-termini and have been identified in chicks, humans, mice, zebrafish and *xenopus*, while the fourth isoform, PITX2d, which lacks the amino-terminal domain and most of the homeodomain, has only been observed in humans.

	83	T97A	125
NP_000316.2 (Human)	AEDPSKKKRQRRQR	T	HFTSQQLQLEATFQRNRYPDMSTREEI
XP_001141234.1 (Chimpanzee)	AEDPSKKKRQRRQR	T	HFTSQQLQLEATFQRNRYPDMSTREEI
XP_001091288.1 (Monkey)	AEDPSKKKRQRRQR	T	HFTSQQLQLEATFQRNRYPDMSTREEI
NP_001091460.1 (Cattle)	AEDPSKKKRQRRQR	T	HFTSQQLQLEATFQRNRYPDMSTREEI
XP_851370.1 (Dog)	TE DPSKKKRQRRQR	T	HFTSQQLQLEATFQRNRYPDMSTREEI
NP_001035967.1 (Mouse)	AEDPSKKKRQRRQR	T	HFTSQQLQLEATFQRNRYPDMSTREEI
NP_001035970.1 (Rat)	AEDPSKKKRQRRQR	T	HFTSQQLQLEATFQRNRYPDMSTREEI
NP_990341.1 (Fowl)	PEDPSKKKRQRRQR	T	HFTSQQLQLEATFQRNRYPDMSTREEI
NP_001138130.1 (Fruitfly)	PKNDKKNKRQRRQR	T	HFTSQQLQLEHTFSRNRYPDMSTREEI
NP_571050.1 (Zebrafish)	NDDPSKKKRQRRQR	T	HFTSQQLQLEATFQRNRYPDMSTREEI
XP_310944.4 (Mosquito)	TKNDKKNKRQRRQR	T	HFTSQQLHELEQTFSRNRYPDMSTREEI

Figure 4. Alignment of multiple pituitary homeobox 2c protein sequences across species. The altered amino acid of p.T97 is completely conserved evolutionarily among various species.

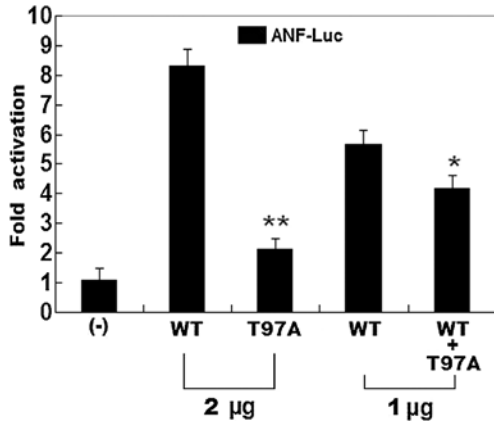


Figure 5. Functional defects associated with pituitary homeobox 2c (PITX2c) mutation. Activation of atrial natriuretic factor (ANF) promoter driven luciferase reporter in Chinese hamster ovary (CHO) cells by PITX2c wild-type (WT) or T97A-mutant, alone or in combination, demonstrated significantly decreased transactivational activity by mutant proteins. Experiments were performed in triplicate, and the mean and standard deviations are shown. \*\*P<0.001; \*P<0.005, when compared with the same amount (2 µg) of wild-type PITX2c.

The unique amino-termini of PITX2a, PITX2b and PITX2c modify their ability to activate transcription in a cell type- and promoter-dependent manner. The homeodomain is responsible for recognizing specific DNA sequences (5'-TAATCC-3'), which is essential for DNA binding, nuclear translocation and interaction with other transcription factors (57). The human *PITX2c* gene maps to chromosome 4q25, which consists of three exons coding for a protein of 324 amino acids (58). PITX2c is expressed asymmetrically in the developing and adult heart, playing a crucial role in normal cardiovascular genesis and maturation (53). The PITX2c mutation of p.T97A identified in the present study is located in the homeodomain; thus, it can be hypothesized that it may affect the transcriptional activity of PITX2c by interfering with its DNA-binding ability.

Previous studies have validated that PITX2c is an upstream regulator of multiple target genes expressed in the heart during embryogenesis, including the gene that encodes ANF (59). Therefore, the functional effect of a PITX2c mutation can be

explored by assay of the transcriptional activity of the *ANF* promoter in cells expressing PITX2c mutant in contrast to its wild-type counterpart. In the present study, the functional characteristics of the novel PITX2c mutation identified in a patient with AF were investigated by transcriptional activity analysis and the results revealed that the mutation was associated with a significantly diminished transcriptional activity on a downstream gene, suggesting that dysfunctional PITX2c caused by mutation is potentially an alternative pathological mechanism of AF.

The association of functionally impaired PITX2c with the enhanced susceptibility to AF may be partially attributable to the abnormal development of the cardiovascular system, particularly the pulmonary venous myocardium (60). The pulmonary venous vessel is sheathed by a layer of myocardium known as the pulmonary myocardial sleeve, which has been implicated in the initiation and maintenance of AF by several presumably electrophysiological mechanisms, including increased automaticity, anisotropic arrangement of the myocardial fibers and delayed conduction that facilitate re-entry (61). It has been verified that PITX2c is abundantly expressed in the atria and pulmonary myocardium and functions as a repressor of the pacemaker lineage gene program by downregulating the sinoatrial nodal gene program, such as short stature homeobox 2 (ShOX2), hyperpolarization activated cyclic nucleotide-gated potassium channel 4 (HCN4) and Cav3.1, and upregulating a gene program characteristic of a working myocardium phenotype, i.e. NKX2-5, Cx40, Cx43, ANP and Kir2.1 (48,51,53,60). In PITX2c knockout mice, a microarray analysis of gene expression profiles revealed that the sinoatrial nodal genes, ShOX2 and HCN4, were upregulated in the atrium, as well as a number of ion channel genes, including KCNQ1 (51). Therefore, PITX2c loss-of-function mutation may contribute to the switch of the atrial and pulmonary myocardium to a sinoatrial node-like phenotype, creating an arrhythmogenic substrate prone to AF.

It has been corroborated that there are some downstream genes transactivated by PITX2c (48) and loss-of-function mutations in several target molecules have been causally involved in AF, including NKX2-5, Cx40, Cx43 and ANF (28,29-32,42,62). Therefore, mutated PITX2c may

predispose to AF by downregulating the expression of such target genes.

In conclusion, to our knowledge, this is the first study on the association of PITX2c loss-of-function mutation with lone AF, which provides novel insight into the molecular mechanisms underlying AF, indicating potential implications for the early prophylaxis and allele-specific treatment of this prevalent type of arrhythmia.

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