Detection of NKX2.5 gene mutations in patients with isolated atrial septal defect

A thesis

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By
Ghada Mohamed Shehata Nor Eldeen

Research assistant
Molecular Genetics and Enzymology Department
National Research Center

Under the supervision of

Prof.Dr/ Hoda Ali Sadek
Prof. of Clinical& chemical Pathology
Faculty of Medicine
Cairo University

Prof.Dr Ibtessam M.Ramzy
Prof .of Human Genetics
Molecular Genetics and Enzymology Dept.
National Research Center

Dr/Ayatallah Amir Nasef
Lecturer of Clinical& chemical Pathology
Faculty of Medicine
Cairo University

Clinical & chemical Pathology Department
Faculty of medicine
Cairo University
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Abstract

Detection of NKX2.5 gene mutations in patients with isolated atrial septal defect

Ghada Mohamed shehata

The objective of this study is to screen for mutations in the NKX2.5 gene in patients affected by isolated non-syndromic atrial septal defects (ASD) in order to assess the role of NKX2.5 mutations in causing these defects.

The study included 25 unrelated patients with isolated non-syndromic ASD. Control group was 10 normal persons who had no personal or family history of any congenital heart diseases. Screening for hot spots for mutations was done using PCR-SSCP (Single stranded conformation polymorphism) and restriction enzyme assay for the analysis of two exons in four fragments(1A, 1B, 2A& 2B). DNA sequencing analysis was done for samples with abnormal electrophoretic mobility in SSCP.

Mutation analysis using restriction enzyme assay identified mutation in 3 out of the 25 cases (12%) (Thr178 Met, Asn188 Lys) in the homeodomain . And polymorphism (silent mutation) in 2 of the 25 cases in exon 1(8%), by DNA sequencing analysis of cases having abnormal migration pattern by SSCP method. It can also be concluded from the current study that, SSCP is not a sensitive method for screening of mutations and direct DNA sequencing and / or restriction enzyme analysis should be used for detection of mutations. Also screening of mutations in NKX2.5 gene in familial cases of ASD is needed to identify a possible diagnostic tool for carrier detection and prenatal diagnosis of CHDs

Key words

-Atrial septal defect - NKX2.5 -Point mutation -Transcription factors

Contents

List of Abbreviations

ACE	:	Angiotensin converting enzyme
ANP	:	Atrial natriuretic peptide
ASD	:	Atrial septal defect
AV	:	atrioventricular
C Tr c	:	Cardiac troponin c
CHD	:	Congenital heart disease
CoA	:	Coarcitation of aorta
CSX	:	Cardiac –specific homeobox
CTAFS	:	Conotruncal anomaly face syndrome
DGS	:	DiGeorge Syndrome
DNA	:	Deoxyribonucleic acid
ECG	:	Electrocardiogram
HD	:	Homeodomain
Irx4	:	Iroquois homeobox gene 4
LV	:	Left ventricle
MEF2	:	Myocyte enhancer factor 2
MRI	:	Magnetic resonance imaging
mRNA	:	Messenger Ribonucleic Acid
NIH	:	National Institutes of Health
NK2-SD	:	NK2 specific domain
NO.	:	Number
OD	:	Optical density
PCR	:	Polymerase chain reaction
PDA	:	Patent ductus arteriosus
PHT	:	pulmonary hypertension
RNA	:	Ribonucleic Acid
RNA pol II	:	Ribonucleic Acid polymerase II
rpm	:	Revolution per minute
RV	:	Right ventricle
SD	:	Standard deviation
SRF	:	Serum response factor
SSCP	:	Single stranded conformation polymorphism
TAE	:	Tris acetate-EDTA
Taq	:	Thermus aquaticus
TBE	:	Tris borate-EDTA
TBX5	:	T box 5
TCT	:	Thrombin-clotting time
TE	:	Tris-EDTA

TGA	:	Transposition of great arteries
TOF	:	Tetrology of fallot
Tris	:	Tris (hydroxymethyl) aminomethane
UV	:	Ultraviolet
VCFS	:	Velo cardio Facial syndrome
VSD	:	Ventricular septal defect
α-MHC	:	Alfa- myocin heavy chain

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Introduction

Cardiac development is a complex biological process, in which several transcription factors have been implicated ***(Fishman,et al.,1997)***, and the susceptibility of the heart to developmental anomalies reflects this complexity ***(Schwartz, et al., 1999)***.The atrial septum is one of the most sensitive cardiac structures to genetic factors . Several lines of evidence have highlighted a role of NKX2.5 transcription factor in septogenesis process ***(Vaughan, and Basson, 2001*)**.

Atrial septal defect (ASD) is one of the most common heart defects , affecting 1 in 1000 live births and , accounting for about10% of congenital heart defects **(*Hoffmann et al.,2002)***.

The homeobox transcription factor NKX2.5 (also known as CSX: cardiac-specific homeobox) is essential for the later stages of heart development and maturation, it is expressed in the early cardiac mesoderm and in heart muscle lineage throughout life . The NKX2.5 gene studies revealed that it has the capabilities of DNA binding, transcriptional activation, protein-protein interactions , and regulation of other transcription factors ***(Tanaka, et al., 1999).***The homeobox transcription factor NKX2.5 contains 2 exons that encode 324 amino acids ***(Fishman, et al., 1997)***and it has been mapped to chromosome 5q34 ***(Shiojima, et al.,1995).*** Mutations in NKX2.5, a member of the NKX2 class of homeobox genes, have been described in autosomal dominant ASD with progressive atrioventricular (AV) block, and in 1-4 % of sporadic ASD patients ***(McElhinney, et al., 2003).*** Most of these mutations occur within homeodomain , a critical protein domain that interacts specifically with DNA , and are associated with conduction anomalies. Numerous heterozygous mutations of NKX2.5 were

identified in patients with congenital heart disease. ***(Benson, et al., 1999).***Atrioventricular conduction disturbance (AV) and atrial septal defect (ASD) were the most common phenotypes ***(Ikeda, et al., 2002)***.

The molecular genetic studies of NKX2.5 gene is based on using primers designed to amplify both exons and intervening intron by using PCR technique. A number of screening methods for detection of sites of mutations in NKX2.5 were used, such as SSCP (single stranded conformational polymorphism) , restriction enzyme assay , and DNA sequencing.

Aim of work:

-The goal of the present study is to detect mutations in the NKX2.5 gene in patients affected by isolated non-syndromic ASD in order to assess the role of NKX2.5 mutations causing these defects.

- Assess the effectiveness of PCR-SSCP method and restriction enzyme assay in detection of mutations causing atrial septal defect.