Mutations in akap genes (a-kinase anchoring proteins) are associated with known r...

Science, Genetics

Abstract

A-kinase anchoring proteins (AKAPs) are a group of structurally varied scaffold proteins which are found to regulate the PKA pathway by localizing the protein kinase A and some specific enzymes at sub cellular level. So far several of the AKAPs have been found to play critical role in the regulation of phosphorylation of several PKA dependent substrates which are necessary for the modulating the cardiac function. Abnormalities in these AKAPs genes have been found to cause various forms of cardiac disease. The most prominent AKAPs are the AKAP9 and AKAP13. Mutations in the AKAP9 gene has been found to be associated with long QT type 11 syndrome. In case of AKAP13, mutations around the gene have been linked to systolic high blood pressure using Genome wide association studies. Although, AKAP9 and AKAP13 have major roles in overall regulation of the cardiac development, so far there only a few studies which have looked into the association of mutations in these genes to be associated with cardiac disease. The present study aims to screen both the AKAP9 and AKAP13 genes for variants which could be linked to the long QT type 11 syndrome and systolic high blood pressure respectively.

Introduction and Literature Review

A-kinase anchoring proteins (AKAPs) are a group of structurally varied scaffold proteins that regulate the PKA pathway by localizing the protein kinase A and some specific enzymes at sub cellular level. The PKA pathway mainly includes phosphatases, phosphodiesterases or another other kinases like the protein kinase C (PKC) or the protein kinase D (PKD). The specific

proteins and the regulatory enzymes which are regulated by the AKAPs make sure that there is efficient control of phosphorylation state of the specific proteins. This would in turn regulate the series of cellular functions which includes the activation of the neurons, the elasticity to the cardiac rhythm and contraction of the cardiac muscles (Scott and Santana, 2010; Carnegie and Burmeister, 2011; Diviani et al, 2011; Perino et al, 2012; Tröger et al, 2012).

In the heart, the phosphorylation state of about three important ion channel proteins are regulated by the AKAP resolved macromolecular complexes. They are:

- The intracellular calcium-release channel
- The L-type calcium channel
- The delayed rectifier Iks potassium channel

There are several members in the AKAP family and all have a conserved protein kinase A domain which is used for anchoring and binding for other signaling components. So far many of the AKAPs have been found to play critical role in the regulation of phosphorylation of several PKA dependent substrates which are necessary for the modulating the cardiac function. It has been found that AKAPs plays a major role in controlling several physiological functions which includes Ca2+ cycling, contraction of the cardiac muscles and action potential duration (Scott and Santana, 2010; Carnegie and Burmeister, 2011; Diviani et al, 2011; Perino et al, 2012; Tröger et al, 2012).

When the heart is subjected to stress or suffers any kind of injury, different kinds of intracellular pathways gets activated which might affect the

handling of calcium, cytoskeleton, and sarcomeric as well as mitochondrial functions. Studies have found that about 13 different types of AKAPs play crucial role in the cardiac myocytes in regulating the intracellular signaling functions which would initiate remodeling of the extracellular matrix, again reactivate the expression of embryonic gene programs and myocyte hypertrophy. The most common AKAPs whose expression is found in the heart are AKAP-LBC(AKAP13), AKAP9 (yotiao), synemin, gravin, BIG2, mAKAP, SPHKAP, ezrin, AKAP18, AKAP95, AKAP95, AKAP79, D-AKAP1/2 and AKAP220 (Scott and Santana, 2010; Carnegie and Burmeister, 2011; Diviani et al, 2011; Perino et al, 2012; Tröger et al, 2012).

Research studies have found that specific mutations in the AKAPs cause several cellular dysfunctions. There has been evidence of mutations in these specific channel proteins which are inherited to be associated with catecholamenergic polymorphic ventricular tachycardia and long QT syndrome. There have also been reports of abnormal expression of the AKAPs to be associated with chronic heart failures, cardiomyocyte hypertrophy, arrhythmias, adaptive response to hypoxia, cancer and disorders associated with the immune system like Human Immunodeficiency Virus syndrome (Scott and Santana, 2010; Carnegie and Burmeister, 2011; Diviani et al, 2011; Chopra and Knollmann, 2011; Troger et al 2012).

For the current study, AKAP9 and AKAP13 have been chosen from the group of AKAPs to study the association with cardiac disease.

A Kinase Anchor Protein 9 (AKAP9): The AKAP9 gene is a member of the AKAP family which is present on the long arm (q) of chromosome 7 (Chr7q21-q22). The exact molecular location of the gene is from the base

pair 91, 570, 188 to 91, 739, 986bp. Studies have found that mutations in the AKAP9 gene are associated with long QT syndrome type 11 (LQT11). This kind of heart disorder is associated with prolonged QT interval on an electrocardiogram (ECG) and polymorphic ventricular arrhythmias. This condition causes sudden unconsciousness and death when an individual undergoes any kind of stress or performs exercise. This usually presents itself as a sudden cardiac death during infancy. The mutations have been found to be inherited in an autosomaldominant manner; however, a definitive correlation between the genotype and the phenotype has not been studied in detail (AKAP9; Chopra and Knollmann, 2011; Troger et al 2012).

A Kinase Anchor Protein 13 (AKAP13): The AKAP13 gene is also known as AKAP-Lbc is a member of the AKAP family. The AKAP13 gene is present on the long arm (q) of chromosome 15 (Chr7q24-q25). The exact molecular location of the gene is from the base pair 85, 777, 817 to 86, 292, 586bp. Several studies have found that AKAP13 has a prominent role in both cardiac cellular function and its pathogenesis. This particular gene has been found to regulate the signal transduction using several protein kinases and also plays a major role in abnormal cardiac hypertrophy which is an essential underlying factor for cardiovascular disease. Studies have found that a SNP (rs11638762), which is present in the GATA-3 binding site located upstream of the AKAP13 gene to be significantly associated with systolic blood pressure. This same SNP has also been replicated in other studies to be associated with high systolic blood pressure. Based on the functional genome wide association studies the AKAP13 which has an important role in the development of cardiac myocytes in mice has been found to regulate the

blood pressure (AKAP13; Hong et al, 2011).

Although, AKAP9 and AKAP13 have major roles in overall regulation of the cardiac development, so far there only a few studies which have looked into the association of mutations in these genes to be associated with cardiac disease. Based on which the present study is undertaken to analyze the following:

- Association of possible mutations in AKAP9 gene to be associated with long QT

syndrome.

- Association of possible mutations in AKAP13gene to be associated with systolic high

blood pressure.

Aim

Although there has been significant progress in elucidating the role played by the family of AKAP proteins in the normal development of the heart as well as the abnormalities associated with heart disease. Several AKAPs have been found to be expressed in the heart of which AKAP9 and the AKAP13 are the primary ones. However, so far studies based on the complete gene screening of both genes have not been carried out. The present study is designed based on case and control groups. This would help in comparing the variants identified in the genes with the normal control group and determine which variants can be associated with the disease condition. The aims of the present study are as follows:

Aim 1: Screening AKAP9 gene in long QT syndrome type 11 patients to identify known

or novel variants which can be associated with the conditions. Although, now

screening of AKAP9 gene is available as a molecular diagnostic test, so far a clear

understanding of the genotype and phenotype relations is not available. By performing the gene screening in the present study on diseased individuals and

normal individuals, one can bring about an understanding between the variants

and the disease.

Aim2: Screening AKAP13 gene in patients with systolic high blood pressure to identify

known or novel variants which can be associated with the conditions. Till date

there have been only a few association studies which involve gene screening of

the AKAP13 gene to look for variants which can be associated with systolic heart

disease. The present study includes complete gene screening of AKAP13 in both

individuals with systolic heart disease and normal individuals. This would help in identifying variants which can be associated with disease condition.

Experimental design

The present study will be conducted using cases (cardiac disease patients) as well as control groups (normal individuals without any cardiac disease). For screening AKAP9 and AKAP13 genes, two separate groups of cases and controls will be considered. They are:

- AKAP9 gene screening:

A total of 5 patients who were clinically diagnosed to have long QT syndrome type 11 will be considered. Similarly for the control group 5 clinically proven normal individuals without any abnormal electrocardiogram or any other cardiac disease will be considered.

- AKAP13 gene screening:

A total of 5 patients who were clinically diagnosed to have high systolic blood pressure will be considered. Similarly for the control group 5 clinically proven normal individuals without high blood systolic blood pressure or any other cardiac disease will be considered.

Materials

After informed consent is taken from all the individuals participating in the study, about 2ml of whole blood samples will be collected in an EDTA vacutainer (purple tube).

Methods

Extraction of genomic DNA from samples using QIAamp DNA extraction Mini kit:

The QIAamp DNA extraction Mini kit are the ideal choice for purification of total DNA from whole blood samples which can be directly used for PCR

amplification. The isolated DNA is free of protein contamination or any PCR inhibitors. The size of the DNA purified using this kit is about 50kb in size which gets easily denatured during PCR and the efficiency of the yield is very high (Qiagen Protocols). The genomic DNA from both the cases as well as the control groups will be purified using QIAamp DNA extraction Mini kit.

Polymerase Chain reaction for the amplification of the AKAP9 and AKAP13 gene in the human genomic DNA using specific primers:

The Polymerase chain reaction (PCR) is a commonly used technique to amplify small quantity of DNA samples in order to make thousands to millions of copies of the similar DNA sequence using a thermal cycler machine. The major steps involved during a PCR process are as follows (PCR: The Basic Theory):

1. Initialization: This is the first step in the PCR cycle which consists of heating the samples in the reaction tube to a temperature of 95 to 96°C for about 1 to 9 min. This step is required for the activation of the DNA polymerase enzyme in the reaction.

2. Denaturation: During denaturation double stranded DNA opens and forms single stranded DNA. The temperature is usually 94°C for about 20 to 30secs.

- Annealing: The primers (forward and reverse) specific for the sense and the antisense strands bond with the DNA template. The temperature is usually between 50 to 60°C for about 20 to 40secs.

- Extension: During this step the Taq DNA polymerase enzyme starts working by adding dNTPs from 5’ to the 3’ end beginning from the point where the

primer has bonded with the DNA template. The temperature is usually at 72°C for about 40 to 45secs.

- Final elongation: This is the last step in the PCR cycle which makes sure that any remaining single stranded DNA are fully extended. The temperature is usually 72°C for about 5 to 15 mins.

- Final hold: When the PCR cycle is complete the samples can be temporarily stored at 4 to 15°C in the thermal cycler until further procedures in this step.

PCR will be performed on all the genomic DNA samples for all the coding exons using primers at their specific annealing temperatures for both AKAP9 and AKAP13 genes.

Qualitative Analysis of PCR product by Agarose Gel Electrophoresis:

One of the most common methods used in the laboratory to analyze PCR fragments is agarose gel electrophoresis. By passing electric current the molecules like the nucleic acids (DNA and RNA) and the proteins become charged. Based on their charge and size the molecules separate on the gel which could be either agarose or polyacrylamide, depending on the molecules to the separated. In order to qualitatively analyze the PCR fragments of both AKAP9 and AKAP13 genes, agarose gel electrophoresis is performed. By performing this method, one can see if all the genomic DNA samples subjected for PCR amplification have been successfully amplified before proceeding to the next step.

Agarose gel DNA purification by Spin Column method:

In many PCR reactions apart from the DNA band of interest non specific bands also gets amplified. In such cases the DNA bands are cut using sterile cutters from the gel and subjected for purification. The PCR fragment in the agarose gel is purified using silica binding principle which is present in the micro spin columns. Approximately about 100bp to 10kg DNA fragments can be purified using this method. The PCR fragments of interest are purified by eliminating the unused components used for setting up the reaction like the primers and dNTPs along with PCR inhibitors when the DNA fragments are washed using specific reagents. The pure PCR fragments which are bound to the silica membrane are incubated after addition of specific buffer and eluted (Qiagen Protocols).

PCR product purification by Spin Column method:

In many PCR reactions only the DNA band of interest is applied without any non specific bands. In such cases, instead of using the agarose gel DNA purification by Spin Column method, the PCR product itself is purified using the same spin column method. Similar to the agarose gel DNA purification method, the PCR fragments of interest are purified by eliminating the unused components in the reaction mixture like the primers and dNTPs along with PCR inhibitors when the DNA fragments are washed using specific reagents. The pure PCR fragments which are bound to the silica membrane are incubated after addition of specific buffer and eluted (Qiagen Protocols).

Sequencing of the AKAP9 and AKAP13 genes:

The most common method used to sequence the region on interest in the genome is Cycle sequencing. The purified PCR fragments of all the coding exons as well as the boundaries of the exon/intron junctions and a part of the 5’ and 3’ untranslated region of both AKAP9 and AKAP13 will be sequenced to identify possible variants present in the patient samples as well as control samples. Both the forward (sense) and reverse (antisense) strands will be sequenced.

Outcome

The outcome of the present study is to identify known and/or novel variant/s in both the AKAP9 and AKAP13 which can in turn be linked as a possible causative factor/s to long QT type 11 syndrome and systolic hypertension conditions respectively. By performing a case-control study one can rule out common variants which might be present in the diseased individuals as well as normal individuals. Only those variants which are present in the affected individuals can be associated with the disease.

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