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Molecular study of Apolipoprotein E gene in familial hypercholesterolemic families

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Key words: Hypercholesterolemia, Apolipoprotein E gene, Single strand confirmation polymorphism, Isoforms

Abstract

Background: Familial hypercholesterolemia (FH) is understood to be one in all the foremost common hereditary disease and critically associated with Coronary heart condition worldwide. FH is taken into account to be caused because of mutations and polymorphisms within the apolipoprotein E (Apo E) cistron. Exaggerated level of density compound protein LDL-C is that the hallmark of this malady.

Methodology: Seven hypercholesterolemic families were chosen for this study. Case history was taken and pedigree was created in person by visiting every family. Exon3 and exon4 regions of ApoE cistron were amplified using polymerase chain reaction (PCR).After successful amplification, both citrons were sequenced. Single strand conformation polymorphism (SSCP) results were obtained to support the different pattern of single strand polymorphism of studied samples.

Results: The sequencing results of probands from all the seven families showed that six out of seven have Apo E three isoform whereas one family showed change within the sequence from T to C at 112 sequence position of processed macromolecule resulted in amino acid that represents it as Apo E4 isoform.

Conclusion: Our findings show that Apo E3 is more prevalent than Apo E4 and other isoforms in studied population of Pakistan.

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Introduction

Familial hypercholesterolemia (FH) is thought to be one in all the inheritable disorders that is said to be caused due to abnormality in metabolism of plasma lipoprotein. FH is usually manifested by an elevated level (LDL)-cholesterol that ends up in several complications like lipid accumulation in vessels of blood provides, premature arterial sclerosis and nice risk of diseases associated with heart [1]. Clinical and molecular characterization of lipid metabolism describes the FH as firs genetic disease [2].

FH is associate degree chromosome attribute, and loosely characterised into 2 classes supported clinical manifestation and phenotypical prevelance of “heterozygous” and “homozygous” form. blood serum LDL-C level is twice and 4 fold higher in heterozygous and homozygous respectively [3]. FH prevalence relating to heterozygous is roughly one in five hundred individuals (10 million world-wide) and therefore the increased serum cholesterol concentration leads to coronary cardiovascular disease [4]. half the people with FH don't survive before the age of sixty attributable to infarction [5]. The homozygous kind of FH is comparatively rare as compared to heterozygous type (1/1000000 live born) and is much more fatal counting on what variety of mutation could occur and results death within the early age of life [6].

Pakistan has additionally a high illness burden of infarction and its risk factors. Coronary artery disease is found to be the underlying reason for infarction within the overwhelming majority from Islamic Republic of Pakistan [7]. It is reported that at the age of 45% of male and 20% of female patients suffers from coronary artery disease (CAD) [2]. The heterozygous variety of FH is treated by numerous ways like regular exercise, intake of assorted medicinal drug medication and fiber made diet .

Methods

Identification and Sampling of FH positive families

Families with a minimum of 2 or additional individual clinically diagnosed with symptom were selected from completely different areas of District Mardan of Khyber Pukhtoon Khuwa and district Lahore of Punjab, Pakistan. Seven families were designated for this study. Case history was taken and pedigree was made personally by visiting every family. Elaborate history was taken from every family to reduce the presence of different abnormalities and environmental causes of symptom. different relatives of the affected family with symptom were conjointly enclosed within the study counting on their willingness and availableness. Informed consent was obtained for taking part within the study.

Blood samples (5 mL) were collected from all the affected people, their traditional siblings, folks to trace the mode of inheritance. The blood samples were collected in 5 mL vacutainer already containing heparin that work as an anticoagulant, the blood samples were kept in ice boxes in real time when their

collection was made and stored in -20^{0}C before DNA extraction.

Pedigrees were drawn on Cyrillic software V.4.0 with the help of data taken from each affected family. At least three or four generation family data as (sibs, cousin marriage, monozygotic twin and sex) was shown by biological symbols.

DNA was extracted by in-organic method and stored at -80^{o}C for later use [8].

PCR for Apo E gene polymorphisms

The complete sequence of two primers Apo E3 and Apo E 4 were selected from the National Center of Biotechnology Information (NCBI) website at URL (http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi). Specific primers for the selected exons of Apo E were designed using Primer3 software web facility [9].

All the primers were optimized on the BioRad Thermocycler. The conditions were set at which the best results were obtained. Amplification reactions were carried out at temperatures and recipe specific for each primer.

Results

A total of seven families with 2 or additional affected people, clinically diagnosed with hypercholesteremia were known from totally different areas of district Mardan and Lahore of Khyber Pukhtoon Khuwa and Punjab respectively. Proband sample of every family were sequenced using genetic analyzer ABI3130xL.

The sequencing results of probands from all the seven families represent that six out of seven have Apo E three isoform whereas one family showed modification within the sequence from T to C at 112 sequence position of processed protein resulted in cystine to arginine that indicate it as Apo E4 isoform. Position of processed amino acid 112 and 158 are 130 and 176 codon respectively in transcript strand of mRNA (Ensembl.org). Our findings show that Apo E3 is more prevalent than other isoforms. Similar findings were also reported by using the restriction analysis[10].

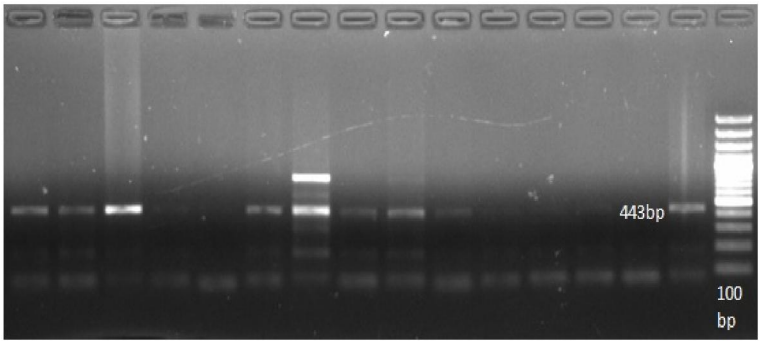

Figure 1: PCR product of primer Apo E3 of 443bp along with 100bp ladder

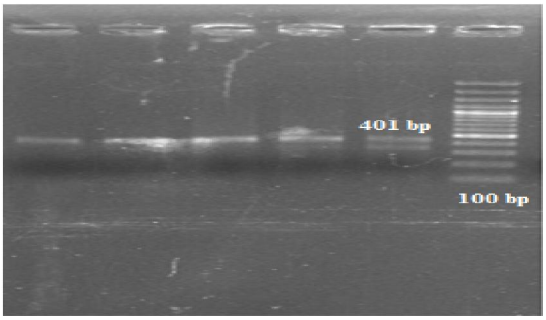

Figure 2: PCR product of primer exon 4 along with 100bp ladder

FH Families	Type	Codon	Sequence	Amino Acid
FH-01	Apo E IV	112	CGC	Arg
		158	CGC	Arg
FH-02	Apo E III	112	TGC	Cys
		158	CGC	Arg
FH-03	Apo E III	112	TGC	Cys
		158	CGC	Arg
FH-04	Apo E III	112	TGC	Cys
		158	CGC	Arg
FH-05	Apo E III	112	TGC	Cys
		158	CGC	Arg
FH-06	Apo E III	112	TGC	Cys
		158	CGC	Arg
FH-07	Apo E III	112	TGC	Cys
		158	CGC	Arg

Table 1: Position of Apo E isoforms and their sequences

SSCP

Single strand conformation polymorphism (SSCP) results were obtained to support the different pattern of single strand with other samples. The sequenced sample of family FH-01 was considered as standard and a single sample from each family was taken to compare the pattern.

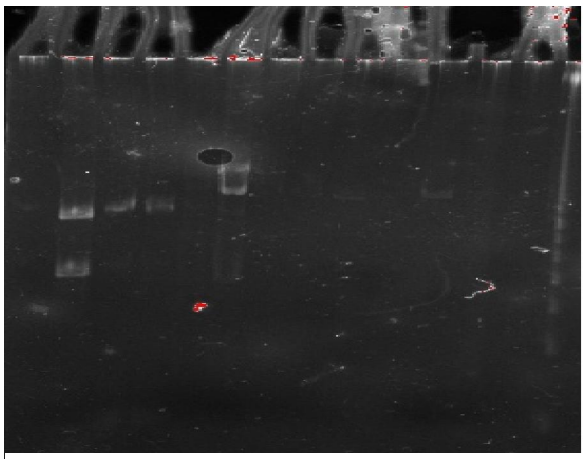

Figure 2: Results of SSCP using polyacrylamide gel electrophoresis. C/F1 represents control/mutated sample of family FH-01 while F2, F3 and F4 represent family FH-02, FH-03 and FH-04 respectively.

Discussion

In present research, we determined the of Apo E polymorphism frequency in seven families of district Mardan of Khyber Pukhtoon Khuwa and district Lahore of Punjab. The sequencing results of probands from all the seven families represent that six out of seven have Apo E3 isoform while one family showed change in the sequence from T to C at 112 codon position of processed protein resulted in cystine to arginine which indicate it as Apo E4 isoform. Position of processed amino acid 112 and 158 are 130 and 176 codon respectively in transcript strand of mRNA (Ensembl.org). Our findings show that Apo E3 is more prevalent than other isoforms. Similar findings were also reported by using the restriction analysis [10]. The prevalence of isoform E3 in six families out of seven families indicates that this is the most prevalent genotype in the selected families of Pakistan

The four genotypes detected were ε3/ε3 (73.9%), ε2/ε3 (6.5%), ε3/ε4 (17.4%) and ε2/ε4 (2.2%). Similar results with matching frequencies of genotypes were reported in one hundred and sixty (160) Lebanese applying the identical molecular methods used here in this study: ε3/ε3 (68.75%), ε3/ε4 (16.25%), ε2/ε3 (13.75%), ε2/ε4 (0.625%), and ε4/ε4 (0.625%) [11]. Another study from Lebanese Republic revealed in 1999 determining the subsequent composition frequencies in a 155 healthy adults ε2/ε3 (12.90%), ε3/ε4 (8.39%), ε3/ε3 (77.42%) and ε2/ε4 (1.29%) [12]. Homozygous ε2/ε2 and ε4/ε4 weren't detected. Absence of homozgousity may be due to low frequency of polymorphism in Lebanese population or low sample size. Yet, Mahfouz and his colleagues found just single case of ε4/ε4 but no case of ε2/ε2 genotype from total subjected Lebanese samples (160), while Almawi and his coworkers failed to notice any of these homozygous genotypes in the 155 healthy subjects investigated [11,12].

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