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Short Communication

Significance of Blood Cellular lxr-α Gene Aberration in Coronary Heart Disease Subjects

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Abstract

Keeping in view our previous finding that unambiguously revealed a significant positive correlation between the expression of mutated Liver X Receptor (LXR)-α gene and the extent of coronary heart disease (CHD), the present study was addressed to explore whether or not this observed blood cellular LXR-α gene aberration is pathognomonic feature of CHD. To detect previously reported blood cellular LXR-α gene aberration restriction digestion was performed with TaaI endonuclease in the LXR-α ligand binding domain derived from the cDNA library of peripheral blood mononuclear cells isolated from different unrelated inflammatory disease group (Rheumatic heart disease, Diabetes, Psoriasis and Tuberculosis) including coronary heart disease. Inheritance of reported blood cellular LXR-α gene aberration was also checked in a family having a higher risk of CHD. Results of our study revealed that LXR-α gene aberration was not only selectively and specifically observed in the blood mononuclear cells derived from CHD patients but also showed a nonmendelian epigenetic inheritance in a family having higher risk of CHD. Based upon these results we propose that blood cellular LXR-α gene aberration may have the potential to act as a noninvasive marker for the early diagnosis of subjects that are at high risk of development of CHD.

Key words: Coronary heart disease, LXR-α, Inflammation, blood marker.

Introduction

Coronary Heart Disease (CHD) is a multifactorial disease with a molecular etiology of gene-gene and gene-environment interaction. Large body of data indicates that conventional risk factors contributes less than 50% in the development of CHD and the studies from identical twins have shown that genetic factors contributes significantly[1], as well as heritability of atherosclerotic phenotype accounts for 40% to 60% of the patients[2]. There is a general recognition of the fact that co-operativity between the lipid peroxidation and inflammation within the arterial wall plays a crucial role in the development of CHD[3, 4]. Recently at the epigenomic level, Liver X Receptor (LXR)-α gene has caught the imagination of researchers across the globe because of its inherent ability to regulate the genes that are known to play crucial role in lipid metabolism and inflammation, the two pathological hallmark of CHD[5,6]. Our recent study revealed a paradoxical relationship between the expression of LXR-α gene, the extent of the coronary occlusion and existence of deregulated LXR-α transcriptome in peripheral blood mononuclear cells derived from CHD subjects[7].

The deregulated LXR-α transcriptome was found to be as a result of three critical mutations in the ligand binding domain (LBD) of LXR-α protein comprising of Asp324, Pro327 and Arg328 which were responsible for inability of this domain to interact with its natural ligands[7]. Keeping in view our findings with real time melting curve analysis that there exist significant positive correlation between the expression of mutated LXR-α gene and the extent of severity of coronary heart disease[7] (Figure 1A) the present study was designed to explore two specific issues:- 1) Whether or not the reported genetic aberration in blood cellular LXR-α gene are CHD specific or common to other inflammatory diseases as well? 2) What is the nature of inheritance of the aberrant LXR-α gene?

Material and Methods

Subject Selection: In the present study we employed the patients suffering from rheumatic heart disease (RHD) (n=15), Diabetes (n=15) with angiographically confirmed normal coronary artery. In addition patients suffering from psoriasis (n=15) and tuberculosis (n=15) were also included. Angiographically confirmed CHD subjects (n=50) were taken as positive control for the previously reported blood cellular LXR-α gene aberration[7]. Due to ethical reasons angiography was not done in subjects suffering from psoriasis and tuberculosis. Further a family (parents with four siblings) with higher risk for CHD was also employed in the present study. Peripheral blood mononuclear cells (PBMCs) were isolated by ficoll hypaque density gradient

method from 5ml of blood drawn from each subject employed in the study with their prior informed consent[8].The demographic and clinical findings of all diseases group have been shown in Tables. A) Based upon melting curve analysis (Dave et al., 2009), correlation between the percent expression of mutated LXR-α mRNA with respect to severity of CHD. Values of "r" show Spearman rank correlation coefficient.

B) Representative 15% polyacrylamide gel stained with ethidium bromide showing the digestion pattern of the amplified region of ligand binding domain of LXR-α derived from different diseased group. L= Ladder, 1= Coronary Artery disease, 2= Rheumatic heart disease, 3= Diabetes, 4= Psoriosis and 5= Tuberculosis.

Table-1
Demographic and clinical findings of the recruited subjects in the study

Disease Group	**No. of Subjects**	**Mean age±S.D.**	**Inclusion Criteria**
Coronary Heart Disease	n=50	48.69±5.94	Angiographically confirmed CHD patient, Severity of CHD was calculated by Gensini Score[10](1->30), Normolipidemic
Rheumatic Heart Disease	n=15	45.26±7.57	According to modified Jones Criteria[11], Angiographically normal coronary arteries
Diabetes	n=15	46.53±7.58	Plasma fasting glucose >200mg/dl, Glycated Haemoglobin >7%, Angiographically normal coronary arteries
Tuberculosis	n=15	48.2±6.14	Sputum acid fast bacilli positive patients
Psoriasis	n=15	48.8±4.85	Patients with mild or moderate psoriasis (Psoriasis Area and Severity Index (PASI) >10) [12]

[Abbreviations: M=male, F= female, HTN= hypertension, Db= diabetes, TC= total cholesterol, LDLC= low density lipoprotein cholesterol, HDLC= high density lipoprotein, TG= triglycerides, RCA= right coronary artery, LM= left main, LAD= left anterior descending, LCX= left circumflex, Prox= proximal, Mid= middle]

Table-2
Demographic and clinical findings of the individuals of a family on a high risk of coronary *heart disease*

Individual	**Age/Sex HTN/Db**	**Lipid Profile** (Values in mg/dl)	**Angiographic finding**
Father	52 M HTN^+ / Db^-	TC=248, LDLC= 165 HDLC= 45, TG= 207	RCA= N, LM= N, LAD= prox 100%, mid 70%, LCX= prox 50%, mid 70%
Mother	45 F HTN^- / Db^-	TC=176, LDLC=107 HDLC= 38, TG= 162	**NIL**
Sibling 1	28 M HTN^+ / Db^-	TC=315, LDLC= 196 HDLC= 48, TG= 339	
Sibling 2	26 M HTN^+ / Db^-	TC=288, LDLC= 209 HDLC= 33, TG= 390	
Sibling 3	22 M HTN^- / Db^-	TC= 275, LDLC= 169 , HDLC= 45, TG= 305	
Sibling 4	20 F HTN^- / Db^-	TC=218, LDLC= 134 HDLC= 38, TG= 229	

Figure legends

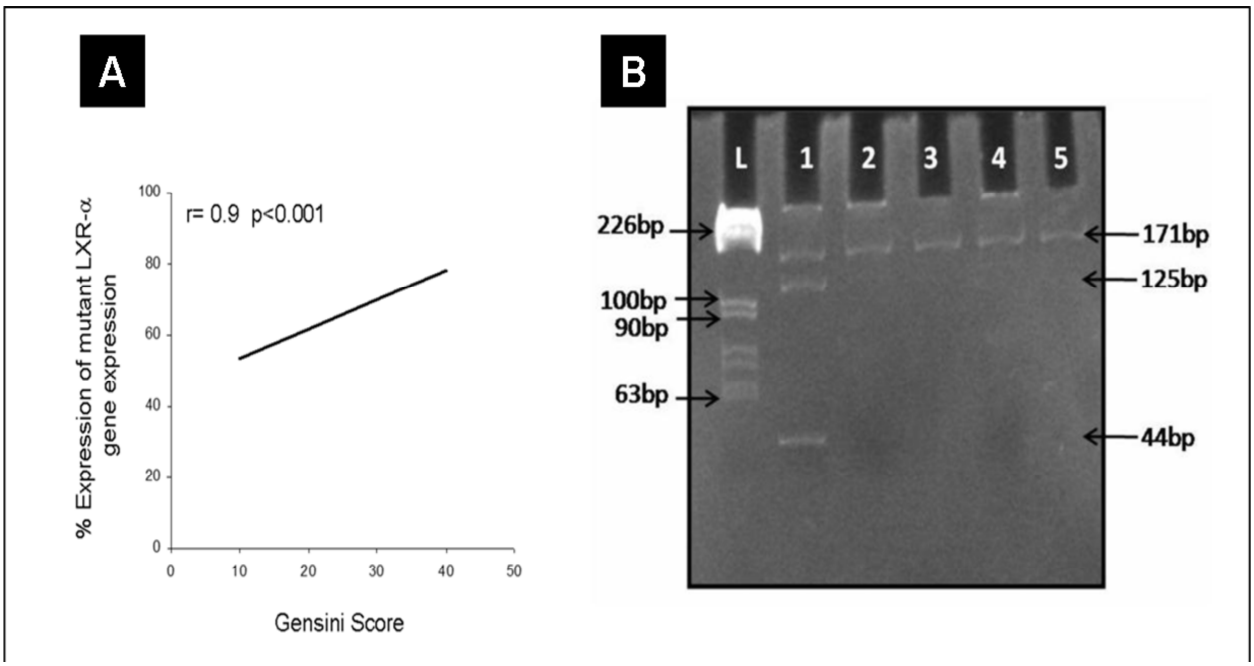

Figure 1
Restriction digestion analysis of LXR-α ligand binding domain by Taa-I
In the subjects suffering from unrelated inflammatory diseases

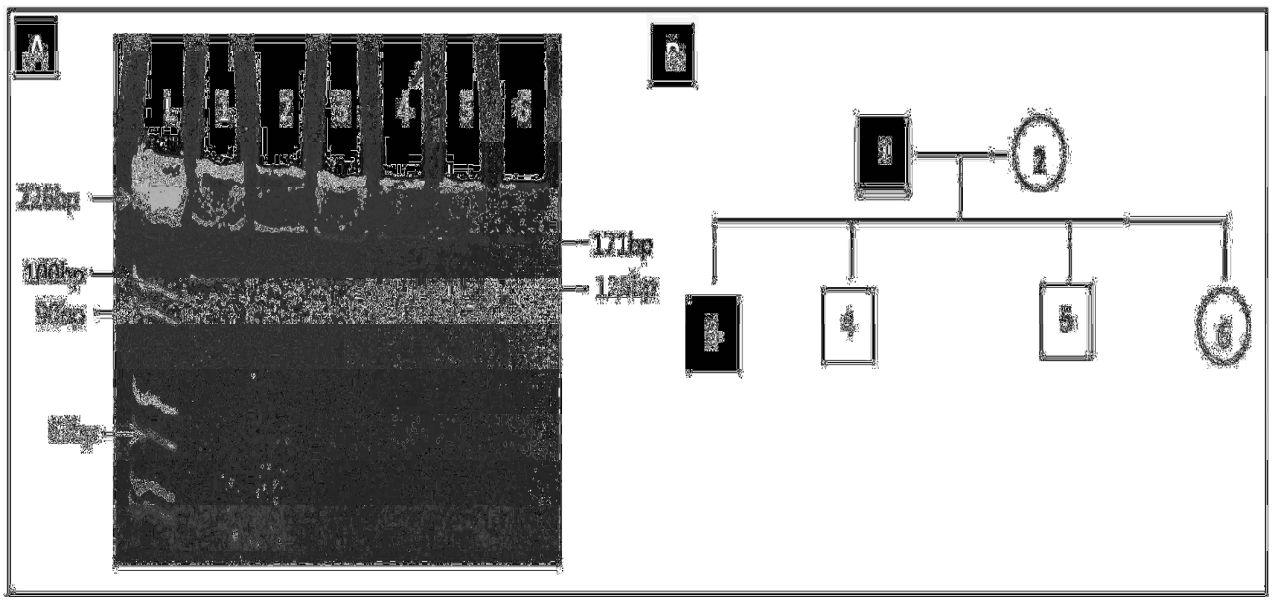

Figure-2
Restriction digestion analysis of LXR-α ligand binding domain by
Taa-I in the individuals of a family on the high risk of coronary heart disease

A) Representative 15% polyacrylamide gel stained with ethidium bromide showing the digestion pattern of theamplified region of ligand binding domain of LXR-α mRNA derived from the individuals of a family at high risk of CHD.

B) Pedigree chart of the family showing the presence of aberrant blood cellular LXR-α mRNA.

Detection of aberrant LXR-α gene: The isolated PBMCs were processed for RNA isolation using standard method[9]. cDNA was synthesized from isolated RNA using Revert Aid^{TM} first strand synthesis kit. LXR-α ligand binding domain (LBD) was amplified by forward primer (5′CAGATTGCCCTGCTGAAGAC3′) and Reverse primer (5′GAACTCGAAGATGGGGTTGA3′) by polymerase chain reaction.

Now the amplified LXR-α LBD (169bp) was subjected to TaaI (recognition sequence ACNGT) restriction digestion. The digested product was resolved by 15% polyacrylamide gel electrophoresis.

Results and Discussion

The results reported here unambiguously showed that the observed blood cellular LXR-α gene aberration, that was earlier found to be the most pathognomonic feature in subjects suffering from CHD, could not be detected in subjects suffering from rheumatic heart disease, diabetes, psoriasis and tuberculosis (Figure 1B). Further in order to explore whether or not observed cellular LXR-α gene aberration is an inheritable genetic disorder, a family having higher risk of CHD was examined. Result of such a study revealed the existence of blood cellular LXR-α gene aberration in father as well as in the eldest son (Figure 2A,B). The results pointed to the fact that the observed LXR-α gene aberration in CHD patients could be as a results of non mendelian epigenetic inheritance.

Conclusion

Keeping in view the fact that inflammation is the common denominator of all the above mentioned unrelated diseases, it was pertinent to note that the observed blood cellular LXR-α gene aberration was a specific feature observed in subjects suffering from CHD. Consequently based on this pilot study, it is not unlikely that the observed blood cellular LXR-α gene aberration could act as a non invasive marker for the detection of subjects/preliminary screening, that are at high risk of susceptibility to CHD, However population based epidemiological studies in different geographical region across the globe are needed to confirm this claim.

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