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Full Length Research Paper

Four novel mutations detected in the exon 1 of MBL2 gene associated with rheumatic heart disease in South Indian patients

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The aim of this study was to determine the genetic variations associated with the mannose-binding lectin 2 (MBL2) gene in rheumatic heart disease (RHD) patients in the Vellore region, South India. This study included 50 patients with RHD and equal number of age and sex matched healthy controls. The genomic DNA was extracted from peripheral blood, to find out the genetic variations if any in MBL2 gene. The exon 1 of MBL2 gene was amplified by polymerase chain reaction (PCR) and then screened with Single Strand Conformation Polymorphism (SSCP) analysis. DNA sequencing was carried out in ABI PRISM® 3730 DNA analyzer. The sequence data were edited as required using the sequence analysing software and sequences were aligned using Autoassembler version 2.0 software. Four novel mutations in four RHD patients in exon 1 of MBL2 gene were observed, (1) 46 G/A (Heteroplasmic mutation) (2) 47 G (deletion), 3) 67 G → A (serine to phenyl alanine) and (4) 96 G (insertion). This is the first report of these novel mutations detected in exon 1 of MBL2 gene of RHD patients in South India. The clinical importance of the study is understanding the genomic nature of every population may show variation in its degree of susceptibility to any environmental insults.

Key words: Rheumatic heart disease, RHD, MBL2 mutation, mannan-binding lectin.

INTRODUCTION

Cardiovascular diseases are the known major and growing contributors to mortality and morbidity in South Asia (Nishtar, 2002). RHD continues to be an important problem in our region, Vellore, South India, though a decline in some other countries has been reported (Jose and Gomathi, 2003; Lalchandani et al., 2000; Krishnaswami et al., 1991). Death rate of coronary heart disease in India rose from 1.17 million in 1990 to 1.59 million in 2000 and is expected to rise to 2.03 million in 2010 (Ghaffar et al., 2004). Almost half of the adult disease burden in South Asia is attributable to non-communicable diseases and the environmental factors that are major determinants. However other factors such as sedentary lifestyles, extreme poverty and inadequate health systems also contribute to the disease.

It is well known that Rheumatic fever (RF), which results from a nonsuppurative sequela of pharyngitis caused by group A streptococcus (GAS) in untreated genetically susceptible hosts, displays a wide spectrum of clinical manifestations including carditis, arthritis, chorea, subcutaneous nodules and erythema marginatum (Bisno, 2000). Recent reports from the developing world have documented Rheumatic Fever incidence rates as high as 206/100 000 and RHD prevalence rates as high as 18.6/1000. The high frequency of RHD in developing world necessitates aggressive prevention and control measures.

GAS displayed strong binding to mannose-binding lectin (MBL) (Neth et al., 2000) encoded by the MBL2 gene in the chromosome region 10q11.1-q21. It plays a major role in innate immunity due to its ability to opsonize pathogens, to enhance their phagocytosis and to activate

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the complement cascade via the lectin pathway (Jack et al., 2001). Inherited insufficiency of MBL that impairs the innate immune function and enhances susceptibility to infection has been shown to be essentially due to three structural variants in exon 1 of the MBL2 gene at codons 52 (C to T), 54 (G to A) and 57 (G to A), corresponding, respectively, to changes of arginine to cysteine (Arg52Cys), glycine to aspartic acid (Gly54Asp) and glycine to glutamic acid (Gly57Glu) in the protein. Any of the three amino acid changes disrupts the collagen helix of the MBL molecule and homozygosity or compound heterozygosity for any of the three alleles results in MBL deficiency (Sumiya et al., 1991).

The worldwide high prevalence of these variants in MBL suggests an apparently ambivalent role for MBL2 in a number of pathogenetic and homeostatic processes (Garred et al., 1994; Ezekowitz, 1998; Saevarsdottir et al., 2004).

Further the knowledge of MBL2 expression may help to understand the pathogenetic mechanisms involved in cardiovascular disease (Best et al., 2009).

It is known that the structure of human populations is relevant in various epidemiological contexts. As a result of variation in frequencies of both genetic and non-genetic risk factors, rates of disease and of such pheno-types as adverse drug response vary across populations (Rosenberg et al., 2002). It was thought it is reasonable to check for the type and nature of MBL2 mutations if any associated with RHD that may provide different type of useful information in a new rare geographical population such as one of South Indian population, Vellore and its surrounding area. As expected four new mutations in exon 1 of MBL2 gene presenting in RHD patients were detected. All those mutations are assigned under four types of novel MBL2 mutation observed for the first time showing the importance of the population genomes in mutation analysis. It is advisable to investigate their usefulness both in basic and clinical studies.

MBL2 gene polymorphism are well established and hence in this study it was tempted to explore the possibility of further genetic variations in the MBL2 gene in rheumatic heart disease (RHD) patients in the Vellore region, South India and this study was undertaken.

MATERIALS AND METHODS

Patients and controls

This study included 50 patients with rheumatic heart disease and equal number of healthy controls. The clinical details of RHD patients are presented in the Table 1.

Both patients and controls provided written consent. Clinically suspected RHD patients were carried out with routine blood investigations, Electrocardiogram (ECG) and Chest X-ray. Confirmation of RHD was done by 2 D colour Doppler (ESAOTE MYLAB 5O X Vision, USA). Patients with at least one deficit valve involvement were included. Cases already underwent surgery or interventional procedures or on drugs were not included in this study. Out of 200 cases referred, 50 were selected for this study.

MBL genotyping

Blood samples were drawn in EDTA vacutainers from the patients. The molecular studies were carried out in all the cases with equal number of control samples.

DNA extraction, quantification and PCR analysis

9 ml of intravenous blood was sampled from all the patients and control by using EDTA coated vaccutainer. The genomic DNA was extracted from peripheral blood by using modified method of Lotery et al. (2000) and standardized at Biomedical Genetics Research Lab at VIT University. Qualitative analysis of DNA was carried out by 0.8% Agarose Gel Electrophoresis and quantification of DNA by using Biophotometer (Eppendorf). Dilutions of DNA were made up to 10 ng/µl concentration by using Tris Ethylene diamine tetra acetic acid (TE) buffer, pH-8.0. The 10 ng/ul of concentration of DNA solution was checked on 0.8% agarose gel. The exon 1 of MBL2 gene was amplified with a pair of primers derived from the published sequence (NCBI accession no. NG_008196 (F -5'-AGGCAGCCAGGCTACTATCA- 3', R (5' TTTGGGGTTGGATGGAAATA -3'). To confirm the amplification of PCR product of exon1 of MBL2 gene, the PCR products were checked by electrophoresis in a 2% agarose gel containing ethidium bromide (0.5 mg/ml) and the bands visualized under UV illumination.

Single strand conformation polymorphism (SSCP) analysis

SSCP was performed by the modified protocol (Orita et al., 1989) that was standardized in Biomedical Genetics Research Laboratory in VIT University, Vellore. Samples were denatured at 95℃ for 5 min and immediately placed on ice for 1 min. 4 µl of PCR product was loaded on 6% polyacrylamide gel. The electrophoresis set up was run at 80 v for 5 h, silver stained and photographed under gel documentation system (Lark innovative, India).

DNA sequencing and analysis

It was carried out using Dideoxy chain termination method (Sanger and Coulson, 1975). The Sequencing was carried out in ABI PRISM® 3730 DNA Analyzer. The sequence data were edited as required using Sequencing Analysis Software™ (Applied Biosystems, USA) and sequences were aligned using Autoassembler version 2.0 software (Applied Biosystems, USA) for identification of mutations/polymorphisms.

RESULTS

The patients (n = 50) undertaken for this study were diagnosed by the clinician and their clinical features are presented in Table 1. In this study, the patients were affected in the first decade and 22 out of 50 patients (44%) showed consanguinity.

The SSCP analysis of the PCR products of the exon 1 of MBL2 gene in RHD patients is presented in Figure 1a.

Out of 50 samples analysed, only 5 (10%) samples showed polymorphism by Single Strand Conformation Polymorphism analysis (SSCP) in MBL2 gene exon1 (RHD 13-1, RHD 19-1, RHD30-1, RHD 31-1, RHD 46-1) and presented in Figure 1a.

Table 1. Clinical and general characteristics of RHD patients

Sl. No.	Individuals studied (case no)	Age at reporting	Sex	Consanguinity	Clinical features (valve defects)
1	RHD-1-1	19	F	Yes	MS/MR
2	RHD-2-1	45	M	Yes	MS
3	RHD-3-1	30	F	Yes	MS
4	RHD-4-1	32	F	Yes	MS
5	RHD-5-1	23	F	No	MS/MR
6	RHD-6-1	11	M	Yes	MR
7	RHD-7-1	21	F	No	MS
8	RHD-8-1	28	F	No	MS
9	RHD-9-1	31	F	No	MS
10	RHD-10-1	30	F	No	MS
11	RHD-11-1	28	F	Yes	MS
12	RHD-12-1	37	F	No	MS/MR
13	RHD-13-1	35	M	No	MS/MR
14	RHD-14-1	50	F	No	MS
15	RHD-15-1	35	M	Yes	MS
16	RHD-16-1	23	F	No	MS
17	RHD-17-1	26	M	No	MR
18	RHD-18-1	48	F	No	MS
19	RHD-19-1	50	F	Yes	MS
20	RHD-20-1	11	F	No	MR with MVP
21	RHD-21-1	34	M	No	MS/MR
22	RHD-22-1	50	F	No	MS/MR
23	RHD-23-1	30	F	Yes	MS/MR
24	RHD-24-1	13	M	Yes	MS/MR
25	RHD-25-1	35	F	No	MS/MR
26	RHD-26-1	32	F	No	MS/MR
27	RHD-27-1	11	M	Yes	MS/MR
28	RHD-28-1	49	M	Yes	MS
29	RHD-29-1	10	M	Yes	MS/MR
30	RHD-30-1	14	F	Yes	MS
31	RHD-31-1	35	F	No	MS/MR.
32	RHD-32-1	15	M	Yes	MS
33	RHD-33-1	39	M	No	MS/MR
34	RHD-34-1	32	F	No	MS/MR
35	RHD-35-1	45	F	Yes	MS/MR
36	RHD-36-1	43	F	Yes	MS
37	RHD-37-1	42	M	No	MS/MR
38	RHD-38-1	40	F	No	MS
39	RHD-39-1	36	M	Yes	MS/MR
40	RHD-40-1	13	M	No	MS
41	RHD-41-1	28	F	No	MS
42	RHD-42-1	26	F	No	MR
43	RHD-43-1	41	M	Yes	MS
44	RHD-44-1	40	F	No	MR
45	RHD-45-1	38	F	Yes	MR
46	RHD-46-1	18	M	Yes	MS
47	RHD-47-1	17	M	No	MR
48	RHD-48-1	50	F	Yes	MS
49	RHD-49-1	19	F	No	MS/MR
50	RHD-50-1	16	M	No	MR

MS, Mitral stenosis; MR, Mitral regurgitation; MVP, Mitral valve prolapsed.

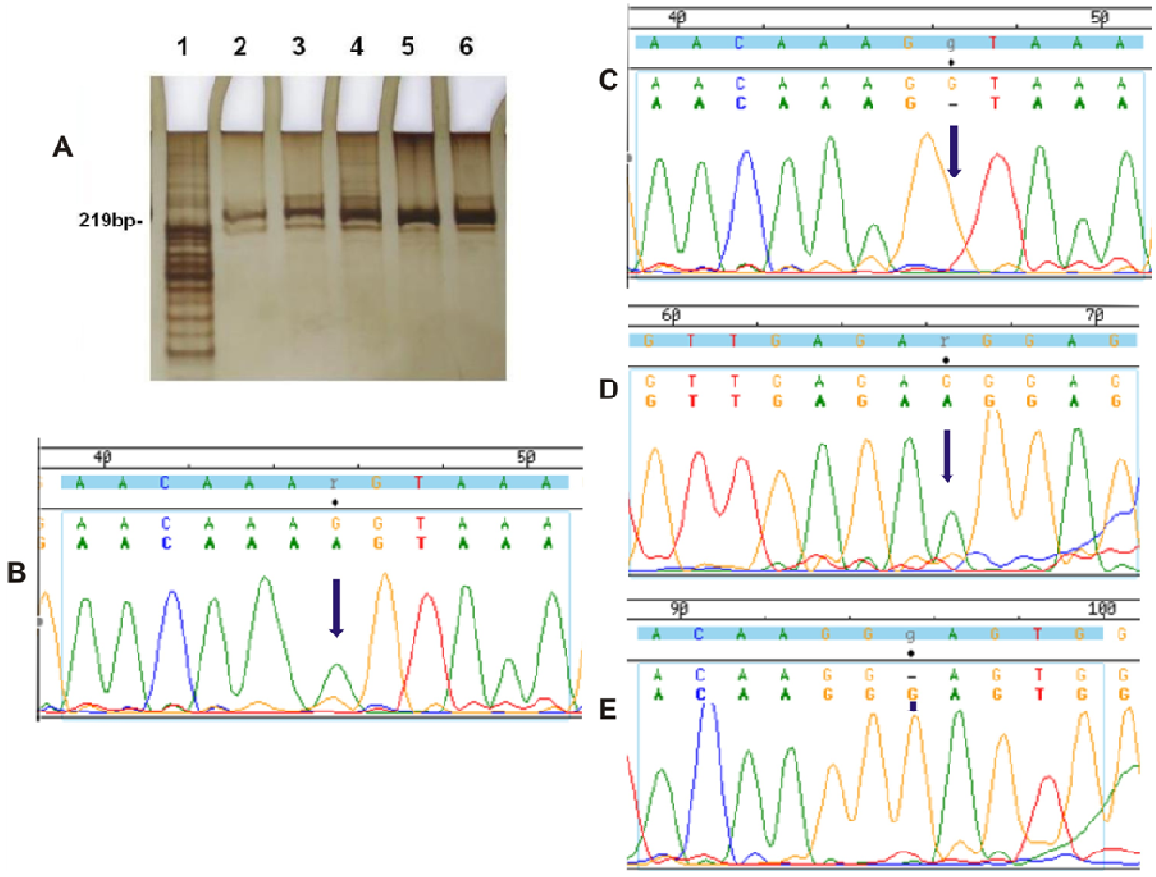

Figure 1. A; SSCP analysis of the PCR products of the exon 1 of MBL2 gene in RHD samples. 1→10bp DNA ladder; 2→RHD 30-1; 3→RHD 31-1; 4→RHD 19-1; 5→RHD 13-1; 6→RHD 46-1. B, C, D and E; Novel mutations observed in exon 1 of MBL2 gene. The reference sequence is aligned on the top of the sequences. The arrow indicates the position of the mutation.

In this study, sequencing of MBL2 gene exon1 for the RHD samples revealed four types novel mutations in 4 RHD patients. The patient (RHD-46) showed G→A heteroplasmic mutation in 46th locus of MBL2 gene, RHD-19 showed guanine nucleotide deletion at 47th locus, one patient (RHD-30) showed nucleotide change from 67 G→A, serine to phenylalanine (UCC→UUC) and RHD-13 patient showed Guanine nucleotide insertion at 96th locus. They are presented in Figures 1 (b, c, d and e) and Table 2.

DISCUSSION

All the children with RHD were affected in the first decade as been similarly observed by Steffensen et al. (2000). One of the common features of RHD cases was mitral stenosis as reported by Schafranski et al. (2004). Mutation screening of exon 1 of MBL2 gene was done by PCR-SSCP analysis for all the samples.

MBL is considered as a modifying molecule in the physiopathology of RF and RHD, presenting a dual role, on one side conferring protection against initial infection by GAS and on the other side provoking inflammatory response in the chronic stage of the disease (Messias et al., 2006). Many factors are certainly involved in the course of RF/RHD, where MBL genotypes as well as MBL protein levels may also play a role.

MBL, an important component of the complement system, plays a key role in innate immunity. As the main role of innate immunity is to restrict the multiplication of infectious agents, deficiency in one of the genes involved in innate immunity may delay or impair the clearance of the pathogens and persistence of the pathogens may trigger the immune system response. Indeed, subjects who are homozygous or compound heterozygous for defective MBL2 alleles or who have low serum MBL suffer from recurrent bacterial and viral infections as children (Turner and Hamvas, 2000) and a subset of them have enhanced risk for autoimmune disorders (Thiel et al., 2006). There is increasing evidence that MBL is also involved in the modulation of disease severity in both infectious and autoimmune diseases (Garred et al., 1997; Garred et al., 2000; Tabona et al., 1995). Steffensen et al.

Table 2. The types of MBL gene mutation observed in RHD patients.

Case no.	Clinical characterestics	Gene/exon	Nucleotide changes	Amino acid changes	Remarks
RHD 13	MS/MR	MBL2, exon 1	96G	Insertion	Novel
RHD 19	MS	MBL2, exon 1	46 G/A	Heteroplasmic mutation	Novel
RHD 30	MS	MBL2, exon 1	67G→A	Serine→Phenyl alanine	Novel
RHD 46	MS	MBL2, exon 1	47 G	Deletion	Novel

MS, Mitral stenosis; MR, Mitral regurgitation.

(2000) reported point mutations in 52, 54 and 57 codons of the exon1 in Caucasian population. However in this study, sequencing of MBL2 gene exon1 for the RHD samples revealed novel mutations in 4 RHD patients. The patient (RHD-46) showed G→A heteroplasmic mutation in 46th locus of MBL2 gene, RHD-19 showed guanine nucleotide deletion at 47th locus, one patient; (RHD-30) showed nucleotide change from 67 G→A, serine to phenylalanine (UCC→UUC) and RHD-13 patient showed nucleotide Guanine insertion at 96th locus.

This study is in line with that of Reason et al. (2006) that had mitral valve lesions; however the patients were Brazilian Caucasians different from our South Indian patients. The worldwide high population prevalence of these variants suggests an apparently ambivalent role of MBL2 in a number of pathogenetic and homeostatic processes (Garred et al., 1994; Ezekowitz, 1998; Saevarsdottir et al., 2004). Further understanding of MBL2 expression may improve our ability to understand and disrupt the pathogenetic mechanisms involved in cardiovascular disease (Best et al., 2009).

Rosenberg et al. (2002) feels that "The structure of human populations is relevant in various epidemiological contexts. As a result of variation in frequencies of both genetic and non-genetic risk factors, rates of disease and of such phenotypes as adverse drug response vary across populations. As expected in this analysis, four novel mutations in exon 1 of MBL2 gene confer a greater susceptibility for developing RHD. These are all novel MBL2 exon 1 mutation observed for the first time in our new geographical area Tamil Nadu in South Indian population. Further, information about a patient's population of origin might provide health care practitioners with information about risk when direct causes of disease are unknown.

Conclusion

Certainly, molecular genetics approach has to be performed for proper medical management and genetic counseling for the RHD patients. MBL replacement therapy might be warranted in the future, MBL deficiency leads to increased susceptibility to disease, MBL replacement could be used to increase resistance to that disease. In an acute infection MBL therapy might, by enhancing the immune response, speed the resolution of disease in MBL-deficient patients. MBL therapy could be used to alter the natural history of chronic diseases (Summerfiled, 2003).

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