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Mutation Screening of MEFV and TNF Gene in Pakistani Patients with Rheumatic Heart Disease: A Case Control-Study

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Abstract

Background: Rheumatic heart disease (RHD) is an inflammatory autoimmune cardiovascular disorder. The disease is highly prevalent in both urban (22 per 1000 individuals) and rural (5.7 per 1000 individuals) areas of Pakistan. The main purpose of this research work was to examine the role of two most widely studied genes, *MEFV* and *TNF* in the susceptibility of RHD in Pakistani patients.

Methods and Materials: In total 360 samples, including 156 clinically diagnosed RHD patients and 204 healthy controls were included in the study. Single strand conformational polymorphism (SSCP) and direct DNA sequencing approach were used to identify the genetic changes in *TNF* exons and hot spots of *MEFV* gene.

Results: No genetic variation in the two genes was detected in this study except a novel mutation (g.G2,096A) in exon 2 of *MEFV* gene. Computational analysis revealed that this mutation (p.S179N) severely affect the three-dimensional structure of the protein and thus probably has a pathogenic role. However, this mutation was identified in two patients only.

Conclusion: Hence, contribution of this mutation is expected to be very small in Pakistani patients. Our results showed a novel mutation with pathogenic effect in a very small proportion of the RHD patients in Pakistan. However, majority of the patients may have mutation outside the hot spot region of *MEFV* gene or there are other susceptibility factors that are contributing toward high prevalence of RHD in Pakistan. Therefore, it is important to screen the complete *MEFV* gene and other genetic susceptibility factors to understand etiology of RHD and thus manage its increasing incidence.

Keywords: Rheumatic heart disease; MEFV; TNF alpha; Autoimmune; Pakistan

Introduction

Mediterranean fever gene (*MEFV*) and tumor necrosis factor (*TNF*) are crucial for inflammatory processes. *MEFV* (protein product, Pyrin) plays important role in regulating inflammatory process, hematopoiesis, oncogenesis and embryonic development [1]. The role of pyrin in inflammation is to direct the movement of white blood cells to the site of inflammation in order to stop or slow inflammatory process. It communicates with other molecules involved in fighting against infection and regulating inflammatory response [2]. Further, involvement of *MEFV* in FMF suggests that this gene may also have a role in susceptibility of RHD [3]. Based on this hypothesis, *MEFV* gene has been screened in RHD patients in few populations based studies with varying results [4,5]. These observations indicate that there could be population specific susceptibility factors for RHD.

Tumor necrosis factor (*TNF*) is an important multifunctional pro-inflammatory cytokine. It is involved in the regulation of different biological functions such as inflammation, cell proliferation, differentiation, apoptosis, lipid metabolism and coagulation [6]. *TNF* gene has shown high level of variability within itself and is located in highly polymorphic region, human leukocyte antigen (HLA). The production of this pro-inflammatory cytokine in response to infection is partly controlled during transcription; for which genetic polymorphisms play an important role. Such as, raised level of TNF is associated with promoter polymorphism (G-308A) in RF patients [7,8]. Therefore, polymorphisms in *TNF* gene have also been explored in different studies as RHD susceptibility factor [9-11]. Despite the fact that Pakistan has the 4th highest prevalence of RHD in the World, scare data about the genetic susceptibility of RHD is available (WHO, 2011). Previously, we studied different polymorphisms in the *IL10*, *IL1RN*, *TNF* and *HLA* [12,13]. Here, we explore the whole exons of *TNF* and hot spot regions of *MEFV* gene.

Materials and Methods

In total, 360 individuals including 156 RHD patients and 204 normal healthy individuals were recruited for the study. RHD patients 127 females and 29 males; mean age 31 ± 14.10 years were collected from three different health institutes; Shifa International Hospital (SIH), Islamabad, Pakistan Institute of Medical Sciences (PIMS), Islamabad and Armed Forces Institute of Cardiology (AFIC), Rawalpindi. All the RHD patients were with mitral valve disease, had history of RF and were in quiescent or chronic phase. They fulfilled the two dimensional M-mode and Doppler echocardiography criteria for mitral valve disease [14].

The control group comprised of 204 healthy individuals 108 females and 96 males, mean age 45 ± 12.7 years with normal echocardiogram and no history of RF. The patients and the controls were matched ethnically. They were from various castes and tribes of Northern Punjab and Khyber Pakhtunkhwa (KPK) in Pakistan. Venous blood samples were collected after informed written consent of the individuals or their legal guardians. The study protocol was approved by respective institutional bioethics committees of IBGE and SIH. DNA was extracted from blood using a standard organic extraction protocol [15].

In order to screen the samples for hot spot regions in exon 2 and exon 10 of *MEFV* and for all the four exons of *TNF* gene primers were designed using web-based program Primer3 (Table 1 supplementary material). PCR was performed using standard protocol and 100 ng of genomic DNA was used in final volume of 25 µl. All the primer sets had the same first step of 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, extension at 72°C for 45 s and 72°C for 10 min, while the annealing temperature and concentration of $MgCl_2$ varied from primer to primer. The PCR products were denatured at 95°C and run on 12% acrylamide gel to detect any conformational change that reflects change in the nucleotide within amplified genomic region [16]. Samples were carefully examined for mobility shift of bands and Sanger sequencing was done wherever required to confirm the sequence change.

In an attempt to predict the impact of this mutation on the structure and thus function of *MEFV*, we predicted the 3-D structure of the wild-type and mutant protein using I-Tasser [17]. STITCH4 database [18] was used to predict functional protein partners. Docking analysis was carried out using Patch Dock server [19,20]. The refinement of first 10 docked complexes obtained through PatchDock was done using FireDock [21]. Representations (2-Dimensional) and analysis of protein-ligand interaction complexes was done using LIGPLOT [22].

Results

Two hot spots; exon2 and 10 of *MEFV* gene and four exons of *TNF-α* gene were screened for polymorphism in RHD samples. Samples were carefully examined for mobility shift of bands and sequencing was done where required but no polymorphism associated with Pakistani RHD patients was observed for these two genes. However a novel nucleotide change was detected in exon 2 of *MEFV* gene at position g.G2,096A (Figure 1). Two female RHD patients were found to carry this mutation in homozygous state; however, the mutation was not detected in any of the control sample. This nucleotide change alters the codon and results in change of amino acid (Serine to Asparagine; p.S179N). The mutation lies between the Pyrin (PYD) and BBox domain (Figure 1c). No other mutations were detected in any of the other study participants.

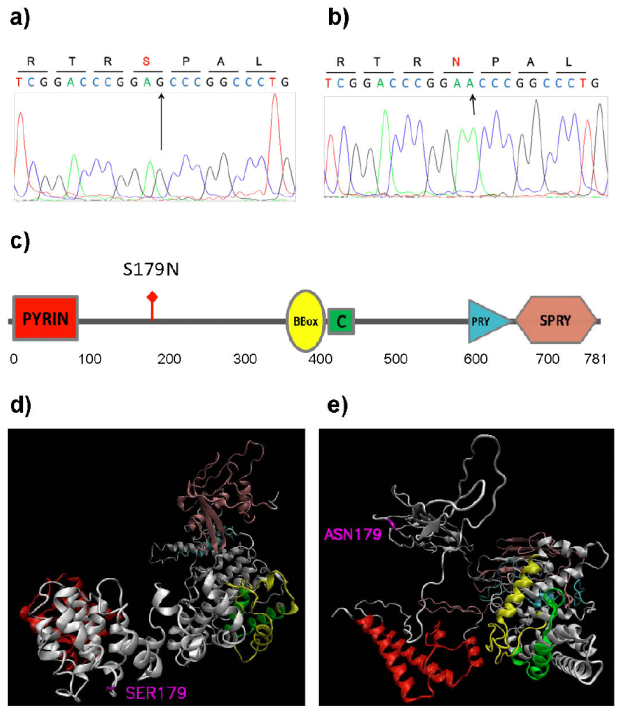

Figure 1: Identification of g.G2,096A (p.S179N) mutation in the *MEFV* gene. Electropherogram shows a 'G' to 'A' nucleotide change in homozygous state in the RHD patient (a, b). This nucleotide change replaces Serine at position 179 to Asparagine and is positioned between PYRIN and BBox domain marked with red line (c). The predication of 3-D structure of wild-type and mutated protein have shown marked differences especially in the positioning of the domains (d, e). The colors of different domains are in accordance with the color as marked in the linear structure. Wild-type MEFV model C-score (0.97), Exp.TM-Score (0.59 ± 0.14), Exp. RMSD (10.6 ± 4.6); S179N mutant MEFV model C-score (1.02), Exp.TM-Score (0.59 ± 0.14), Exp. RMSD (10.8 ± 4.6).

Model prediction and docking analysis confirmed that mutation altered the folding and conformation of MEFV protein. Figure 2 shows 3-dimensional models for wild and mutant proteins. Protein ligand PSTPIP1 (Proline-Serine-Threonine Phosphatase Interacting Protein 1) with maximum interaction score (0.995) with MEFV was selected for docking analysis in reference to results obtained through Stitch4 database. It is involved in regulation of the actin cytoskeleton. It may regulate the WAS actin-bundling activity. Bridges the interaction between ABL1 and PTPN18 leading to the ABL1 de-phosphorylation. It plays a role as a scaffold protein between PTPN12 and WAS and allows PTPN12 to dephosphorylate WAS. It also has the potential to physically couple CD2 and CD2AP to WAS. Wild and mutant types of MEFV were docked with PTPN12 to study the docking interaction and mutation effect. Figure 2 shows three dimensional visualization of docking complexes using viewer ite. Ligplot results for the docking interactions of wild and mutant type MEFV with ligand PTPN12 are shown in Figure 3 part A and B, respectively. Residues involved in hydrogen bonding and hydrophobic interactions during docking are given in Table 1. Mutation although altered the single nucleotide but it caused huge impact on structure and conformation of protein as

compared to normal protein. This can be well observed through differences in number and position of residues involved in both types of docking interactions. This is due to alteration of active site after mutation which effected protein's interaction with ligand and in turn functions of protein.

Receptor-Ligand	Hydrogen Bond Interactions		Hydrophobic Interactions	
	Ligand Residues	Receptor Residues	Ligand Residues	Receptor Residues
Wild MEFV - PTPN12	Asp23, Ser68	Gln198, Arg737	Phe54, Ser67, Gly69	Leu194, Glu195, Gly196, Gly197, Ala736,
Mutated MEFV - PTPN12	Gln9, Arg12, Asn49,	Asp103, Asn113, Asp170	Gly4, Ser5, Gly7, Ala29, Gly30, Ile36, Leu37, Glu38, Glu40, Asp41, Trp43, Thr45, Phe54, Lys62, Leu63, Ser64, Gly65, Pro66, Ser67, Ser68, Gly69,	Leu10, Ser38, Pro41, Arg42, Met53, Thr101, Asp102, Ala105, Ala106, Ser108, Ser109, Leu110, Gly111, Glu112, Lys114, Pro115, Thr326, Leu396, Ile397, Trp626, Leu770, Ile772

Table 1: Receptor (MEFV) and Ligand (PTPN12) residues involved in interactions.

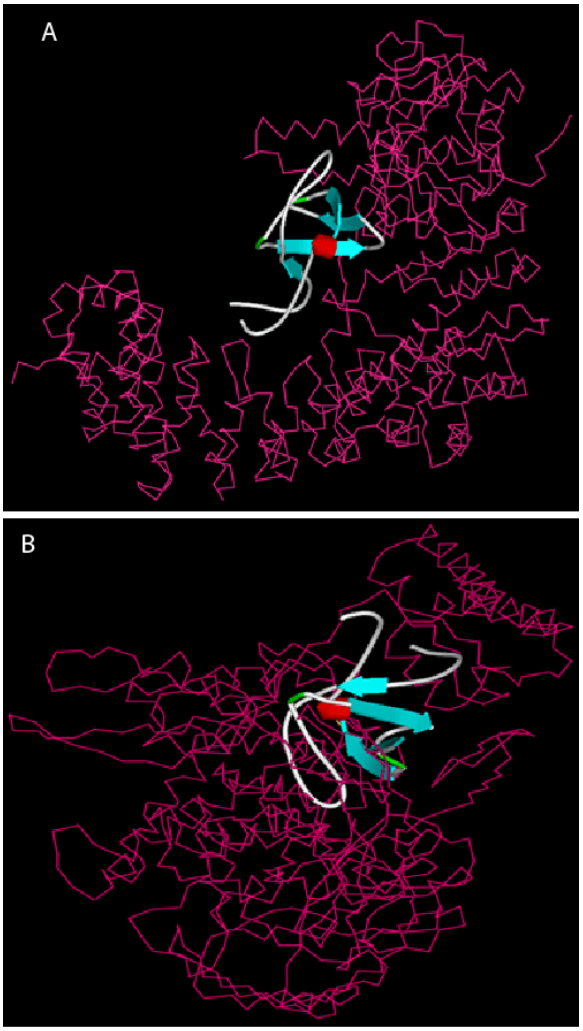

Figure 2: Visualization of docking complex. **A)** MEFV with PTPN12 **B)** Mutant MEFV with PTPN12. Receptor shown with Ca wire display style and pink color, ligand: schematic display style.

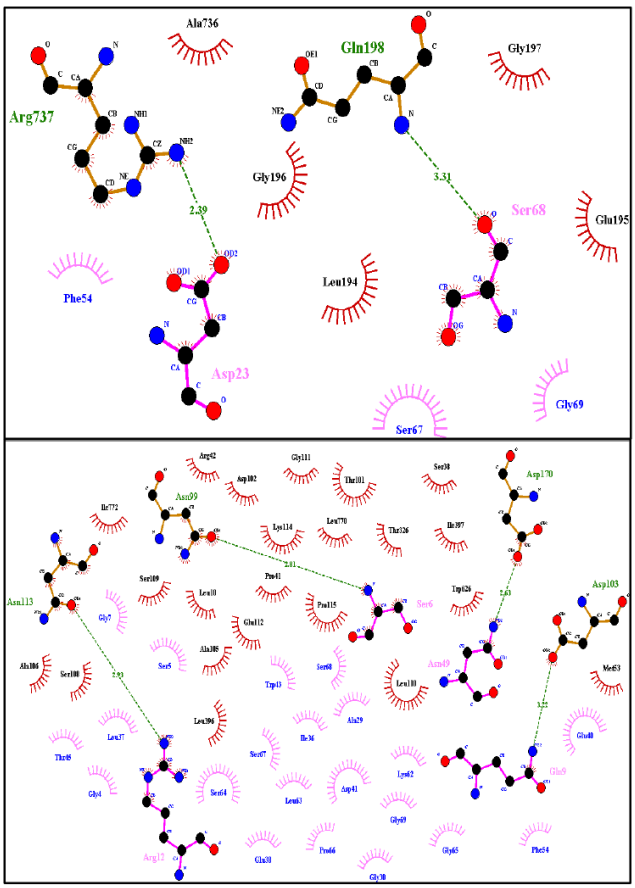

Figure 3: 2D Ligplot representation of Docking Interaction. **A)** Normal MEFV with PTPN12, **B)** Mutant MEFV with PTPN12.

Discussion

MEFV and *TNF* gene product play a key role in immune system and there malfunction through any change in their gene results in the abnormal immune response. *MEFV* gene product pyrin is involved in the regulation of inflammation, variants of *MEFV* gene alters its

function and results in certain inflammatory and autoimmune disorders [23,24]. In a study on 27 Turks RHD patients MEFV mutations were screened and the result showed that the MEFV mutations gene frequency was four times greater in RHD patients than in the normal controls [4,5].

Our result differs from this study as we found no mutation or polymorphism associated with RHD in Pakistani population. There could be several reasons for this discrepancy, one of these is that our group is considerably large n=156 as compared to their 27 patients, secondly the RHD samples they used were relatively of young age ranging from 7 and 18, while our patients mean age range was from 31 ± 14.10 years. Another reason might be that they used sequencing technique and we used SSCP technique. Differences in geographical back ground can also be another reason. The mutations which were studied in Turks occurred in FMF patients, it might be suggested here that our study gives evidence that no association exists between FMF and RF. A novel nucleotide change was detected in exon 2 of *MEFV* gene at position g.G2,096A which is positioned between the Pyrin (PYD) and BBox domain in our study. The PYD is involved in inflammation and apoptosis [25]. It interacts with other proteins like NLR (Nod-like receptor) and ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) to form a large multiprotein complex known as inflammasome [26]. The inflammasomes activates inflammatory pathways and is crucial for host defense against pathogens. Mutations in the PYD domain can influence the interaction of pyrin with ASC and alters the activation of inflammasomes thus leading to auto-inflammatory disorders. Although the mutation detected in this study (p.S179N) is not present in PYD domain; however, it greatly altered the overall 3-D structure of the protein and may potentially influence the formation of inflammasomes. Docking analysis showed interaction site in mutated protein changes which effected protein's interaction with ligand and in turn function of protein. The commonly studied *MEFV* mutation p.E148Q shows some changes in the PRY and SPRY domain (Figure 1, Supplementary material). These minor structural effects in p.E148Q mutant protein are in concordance with the previous findings and therefore exhibit inconclusive phenotypic effect [27,28]. Considering the impact of p.S179N novel mutation on 3-D structure of *MEFV*, it is expected that this mutation may have stronger phenotypic effect compared to the reported mutation (p.E148Q) in the proximity. The p.S179N mutation was detected in two patients only and the rest of the patients did not show any mutation in the 2^{nd} or 10^{th} exon of the *MEFV* gene.

TNF plays an important role in initiation, maintenance and regulation of immune system. Its role in various disease etiologies has been studied and is well documented [29].The level of this important cytokine varies according to the disease conditions. Several promoter polymorphisms are detected in it, which are said to be associated with susceptibility to different diseases including RHD. [13,29]. Due to diverse action and important location of the TNF-α, we designed the primers for its 4 exons to see whether any polymorphism in its gene is associated with etiology of RHD among Pakistani Patients but we were unable to find any polymorphism within this region associated with the disease, to our knowledge this was the first time when the TNF-α exons are screened in RHD patients for genetic variations.

Limitations

Our study has some limitations including small sample size and use of SSCP which is not advanced molecular biology technique like Sequencing.

Conclusion

Our results show that p.S179N novel mutation has pathogenic effect in a very small proportion of the RHD patients in Pakistan. However, majority of the patients may have mutation outside the hot spot region of *MEFV* gene or there are other susceptibility factors that are contributing toward high prevalence of RHD in Pakistan.

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Conflict of Interest

All the authors declare that they have no conflict of interest regarding the publication of this manuscript.

References

1. Centola M, Chen X, Sood R, Deng Z, Aksentijevich I, et al. (1998) Construction of an approximately 700-kb transcript map around the familial Mediterranean fever locus on human chromosome 16p13.3. Genome Res 8: 1172-1191.
2. Pras M (1998) Familial Mediterranean fever: from the clinical syndrome to the cloning of the pyrin gene. Scand J Rheumatol 27: 92-97.
3. Tekin M, Yalçinkaya F, Tümer N, Cakar N, Koçak H (1999) Familial Mediterranean fever and acute rheumatic fever: a pathogenetic relationship? Clin Rheumatol 18: 446-449.
4. Tutar E, Akar N, Atalay S, Yilmaz E, Akar E, et al. (2002) Familial Mediterranean fever gene (MEFV) mutations in patients with rheumatic heart disease. Heart 87: 568-569.
5. Simsek I, Koz C, Basar N, Sari I, Erdem H, et al. (2011) Mediterranean fever (MEFV) gene mutation frequency is not increased in adults with rheumatic heart disease. Clin Rheumatol 30: 491-495.
6. Bazzoni F, Beutler B (1996) The tumor necrosis factor ligand and receptor families. N Engl J Med 334: 1717-1725.
7. Miller LC, Gray ED, Mansour M, Abdin ZH, Kamel R, et al. (1989) Cytokines and immunoglobulin in rheumatic heart disease: production by blood and tonsillar mononuclear cells. J Rheumatol 16: 1436-1442.
8. Sallakci N, Akcurin G, Köksoy S, Kardelen F, Uguz A, et al. (2005) TNF-alpha G-308A polymorphism is associated with rheumatic fever and correlates with increased TNF-alpha production. J Autoimmun 25: 150-154.
9. Settin A, Abdel-Hady H, El-Baz R, Saber I (2007) Gene polymorphisms of TNF-alpha(-308), IL-10(-1082), IL-6(-174), and IL-1Ra(VNTR) related to susceptibility and severity of rheumatic heart disease. Pediatr Cardiol 28: 363-371.
10. Mohamed AA, Rashed LA, Shaker SM, Ammar RI (2010) Association of tumor necrosis factor-alpha polymorphisms with susceptibility and clinical outcomes of rheumatic heart disease. Saudi Med J 31: 644-649.
11. Zheng RL, Zhang H, Jiang WL (2014) Tumor necrosis factor-alpha 308G>A polymorphism and risk of rheumatic heart disease: a meta-analysis. Sci Rep 4: 4731.
12. Rehman S, Akhtar N, Ahmad W, Ayub Q, Mehdi SQ, et al. (2007) Human leukocyte antigen (HLA) class II association with rheumatic heart disease in Pakistan. J Heart Valve Dis 16: 300-304.

13. Rehman S, Akhtar N, Saba N, Munir S, Ahmed W, et al. (2013) A study on the association of TNF-α(-308), IL-6(-174), IL-10(-1082) and IL-1Ra(VNTR) gene polymorphisms with rheumatic heart disease in Pakistani patients. Cytokine 61: 527-531.
14. Braunwald E, Zipes DP, Libby P (2001) Heart Disease: A text book of Cardiovascular Medicine.W. B. Saunders Company, Philadelphia.
15. Sambrook J, Russel DW (2001) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.
16. Xie T, Ho SL, Ma OC (1997) High resolution single strand conformation polymorphism analysis using formamide and ethidium bromide staining. Mol Pathol 50: 276-278.
17. Zhang Y (2008) I-TASSER server for protein 3D structure prediction. BMC Bioinformatics 9: 40.
18. Kuhn M, Szklarczyk D, Franceschini A, von Mering C, Jensen LJ, et al. (2012) STITCH 3: zooming in on protein-chemical interactions. Nucleic Acids Res 40: D876-880.
19. Schneidman-Duhovny D, Inbar Y, Nussinov R, Wolfson HJ (2005) PatchDock and SymmDock: servers for rigid and symmetric docking. Nucleic Acids Res 33: W363-367.
20. Andrusier N, Nussinov R, Wolfson HJ (2007) FireDock: fast interaction refinement in molecular docking. Proteins 69: 139-159.
21. Mashiach E, Schneidman-Duhovny D, Andrusier N, Nussinov R, Wolfson HJ (2008) FireDock: a web server for fast interaction refinement in molecular docking. Nucleic Acids Res 36: W229-232.
22. Wallace CA, Laskowski AR, Thornton MJ (1995) LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng 8: 127-134.
23. Park HH (2012) PYRIN domains and their interactions in the apoptosis and inflammation signaling pathway. Apoptosis 17: 1247-1257.
24. Gershoni-Baruch R, Brik R, Zacks N, Shinawi M, Lidar M, et al. (2003) The contribution of genotypes at the MEFV and SAA1 loci to amyloidosis and disease severity in patients with familial Mediterranean fever. Arthritis Rheum 48: 1149-1155.
25. Taniguchi S, Sagara J (2007) Regulatory molecules involved in inflammasome formation with special reference to a key mediator protein, ASC. Semin Immunopathol 29: 231-238.
26. Ece A, Çakmak E, Uluca Ü, Kelekçi S, Yolbaş İ,et al. (2014) The MEFV mutations and their clinical correlations in children with familial Mediterranean fever in southeast Turkey. Rheumatol Int 34: 207-212.
27. Lidar M, Shinar Y, Goldberg M, Ben-Zvi I, Langevitz P, et al. (2013) E148Q MEFV mutation carriage and longevity in individuals of Ashkenazi origin. Immunol Res 56: 371-375.
28. Naimushin A, Lidar M, Ben Zvi I, Livneh A (2011) The structural effect of the E148Q MEFV mutation on the pyrin protein: a study using a quantum chemistry model. Isr Med Assoc J 13: 199-201.
29. Qidwai T, Khan F (2011) Tumour necrosis factor gene polymorphism and disease prevalence. Scand J Immunol 74: 522-547.