Short Communication

Report of a recurrent mutation in ARS (component B) gene with severe Mal de Meleda in a large consanguineous Pakistani family

Sadia Nawaz[1], Muhammad Tariq[2], Aysha Azhar[3], Mahmood Rasool[4], Syeda Marriam Bakhtiar[5], Ilyas Ahmad[6], Shoaib ur Rehman[7], Muhammad Jameel[8], Tahir Naeem Khan[9], Shehla Anjum Baig[10], Joakim Klar[11], Niklas Dahl[12], Shahid Mahmood Baig[13]

ABSTRACT

Objective: To characterize the disease causing mutation in a large consanguineous Pakistani family with severe Mal de Meleda (MDM) or keratosis palmoplantaris transgrediens, a rare autosomal recessive skin disorder.

Methodology: Single nucleotide polymorphism (SNPs) genotyping was performed using the GeneChip Mapping 250K array (Affymetrix). Homozygosity mapping and sorting of genomic regions were performed with dedicated software called AutoSNPa. Selected regions were further investigated by genotyping with microsatellite markers derived from known and novel polymorphic repeats. Two-point LOD score calculation was performed by using the MLINK of Fastlink computer package. All three coding exons of ARS (component B) gene were amplified by PCR and sequenced.

Conclusion: Sequencing of all the coding exons of ARS (component B) gene in the affected individuals revealed a recurrent missense mutation in exon 3 at base pair 256 from Guanine to Alanine (256G>A) and as a result the amino acid Glycine is replaced by Arginine at position 86 (G86R). This finding will facilitate control of affected MDM births in the Pakistani families.

KEY WORDS: Mal de Meleda (MDM), Palmoplantar keratoderma (PPK), ARS (component B) gene.

Pak J Med Sci April - June 2011 (Part-II) Vol. 27 No. 3 686-689

How to cite this article:

Nawaz S, Tariq M, Azhar A, Rasool M, Bakhtiar SM, Ahmad I, et al. Report of a recurrent mutation in ARS (component B) gene with severe Mal de Meleda in a large consanguineous Pakistani family. Pak J Med Sci 2011;27(3):686-689

INTRODUCTION

Mal de Meleda (MDM, OMIM 24300) also named as keratosis palmoplantaris transgrediens of Siemens is a rare inflammatory skin disease with autosomal recessive transmission. It is characterized by transgressive palmoplantar keratoderma, keratotic skin lesions, perioral erythema, brachydactyly and nail abnormalities. The prevalence of MDM in the general population is estimated to be 1 in 100,000 whereas in Meleda island of Yugoslavia it is approximately 1 in 200 births.[1,2] MDM usually shows an age-related progressive course, and the range and severity of symptoms may vary from case to case. No other organ is involved by the pathologic process. On histopathological examination, hyperkeratosis, acanthosis, and foci of parakeratosis are seen.[3]

The gene responsible for MDM has been mapped to chromosome 8q24.3.[4] The mutations in ARS (Ares-Serono, component B) gene are found to cause the disease in different populations.[5] ARS gene is found within the cluster of Ly6 homologous human genes,

Correspondence:

Shahid Mahmood Baig,
Head Health Biotechnology Division,
Group Leader and Principal Scientist,
Human Molecular Genetics Laboratory,
National Institute for Biotechnology and
Genetic Engineering (NIBGE),
P. O. Box 577, Jhang Road,
Faisalabad, Pakistan.
E-mail: shahidbaig@nibge.org
shahid_baig2002@yahoo.com

* Received for Publication: January 20, 2011

* Accepted: May 18, 2011

encodes secreted lymphocyte antigen-6/urokinase/type plasminogen activator receptor-related protein-1 (SLURP-1), a secreted epidermal neuromodulator involved in epidermal homeostasis and inhibition of TNF-alpha release. SLURP-1 act as a signal in the normal growth and development of palmoplantar keratinocytes by modulating the intracellular calcium content of keratinocytes.[6]

Here, we report a large consanguineous Pakistani family with multiple affected births having characteristic clinical symptoms of autosomal recessive severe MDM phenotype and application of various molecular tools for gene mapping and identification of the pathogenic mutation.

METHODOLOGY

Patients: A large consanguineous family from central Punjab Pakistan with autosomal recessive severe Mal de Meleda (MDM) or keratosis palmoplantaris transgrediens phenotype having 11 affected individuals was ascertained. Family pedigree was drawn with informed consent and all available members underwent extensive clinical examination by a dermatologist. All affected individuals are the offspring of consanguineous marriages (Fig.1) and presented with the severe MDM phenotype. The patients presented with Palmoplantar keratoderma (PPK) that appeared soon after birth, rapidly followed by extension to the sides and dorsa of the feet and hands alongwith thickening of the skin. The transgressive hyperkeratosis severity increased with age and resulted in the characteristic 'glove and stocking' distribution. Nail dystrophy with subungual hyperkeratosis were also observed (Fig.2). The keratoderma resulted in reduced movements and mobility of hands and feet. Perioral involvement was also observed. Nevertheless variable severity was noted among patients due to age differences.

Genotyping, linkage and mutational analysis: Blood samples were taken from 9 affected and 6 normal individuals after informed consent, institutional approval and adherence to the principles of Helsinki Declaration. Genomic DNA was extracted by using standard protocol. Single nucleotide polymorphism (SNPs) genotyping was performed with samples from six affected individuals in the family using the GeneChip Mapping 250K array (Affymetrix) with NSP1 enzyme according to the manufacturers protocol. Homozygosity mapping and sorting of genomic regions were performed with dedicated software called AutoSNPa. Homozygosity cutoff were selected to be >30 SNPs. Selected regions were further investigated by genotyping all members of family with microsatellite markers derived from known and novel polymorphic repeats. Microsatellite markers were amplified by polymerase chain reaction (PCR) with incorporation of fluorescent labels. For fluorescent labeling, we modified the forward primers with the fluorescent labels carboxyfluorescein (FAM) or hexa-chloro-6-carboxy-fluorescein (HEX). Two-point LOD score calculation was performed by using the MLINK of Fastlink computer package with inbreeding loops broken.[7] For the analysis, autosomal recessive mode of inheritance with complete penetrance, equal female to male recombination rate and a disease allele frequency of 0.001 was assumed. Sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Invitrogen, San Diego, USA) on an ABI PRISM 3700 sequencer (Applied Biosystems). The chromatograms were analysed using Sequencher v.4.1.2 (Gene Codes Corporation). The primers and the sequencing conditions are available upon request. All three coding exons of ARS (component B) gene were amplified and sequenced using sequencing conditions and primers described previously.[8]

RESULTS

All the homozygous regions obtained after 250K SNP analysis, were tested with highly polymorphic microsatellite markers. The linkage to all the regions

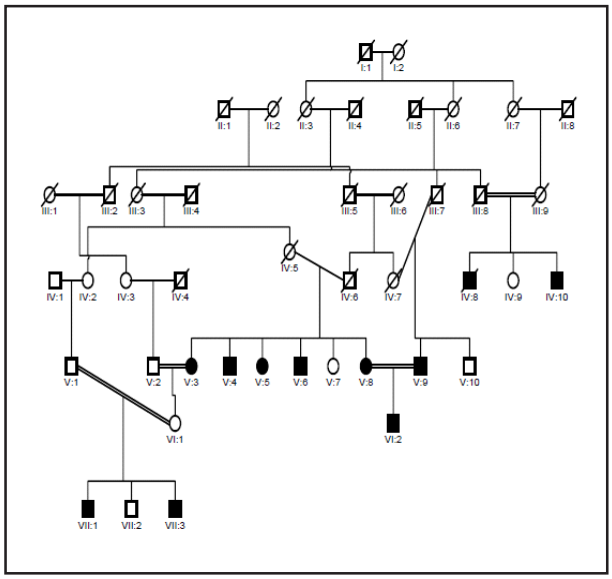

Fig-1: A large consanguineous Pakistani family with severe Mal de Meleda (MDM) or keratosis palmoplantaris transgrediens having 11 affected individuals showing autosomal recessive inheritance pattern.

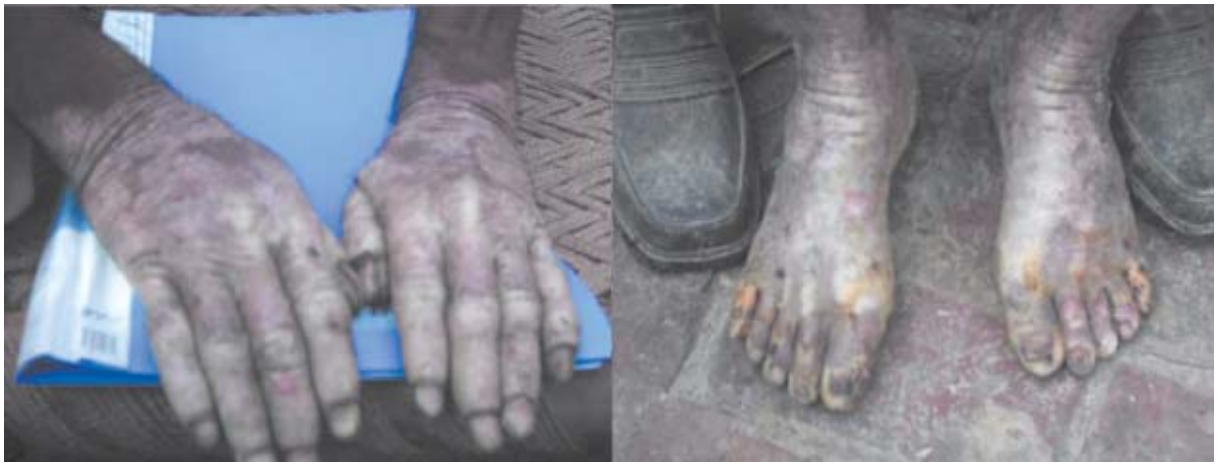

Fig-2: An affected individual with severe Mal de Meleda (MDM) or keratosis palmoplantaris transgrediens extending to the sides and dorsa of the feet and hands alongwith thickening of the skin. The transgressive hyperkeratosis severity increased with age and resulted in the characteristic 'glove and stocking' distribution. Marked nail dystrophy with subungual hyperkeratosis is also visible.

was excluded except one at chromosome # 8 with microsatellite marker D8S161. All the affected individuals shared the same allele in homozygous state. The normal individuals were heterozygous for the marker allele. Two-point LOD score of 3.92 was obtained which further validate the evidence of linkage to this region which contains the ARS (component B) gene on chromosome 8qter.[9] Further sequencing of all the coding exons in two affected individuals revealed a recurrent missense mutation in exon 3 at base pair 256 from Guanine to Alanine (256G>A) and as a result the amino acid Glycine is replaced by Arginine at position 86 (G86R).

DISCUSSION

MDM is a rare autosomal recessive disorder characterized by abnormal thickening of the skin of palms and soles with sharp demarcation that appears soon after birth and progresses with age on to the dorsal surface of hands and feet. The ARS (component B) gene is found to be mutated and underlie the disease phenotype. The ARS (component B) gene encodes secreted SLUP-1, which on immunostaining showed expression in the skin, gums, exocervix and esophagus regulated by retinoic acid, epidermal growth factor, and interferon-γ.[10] Association of SLURP-1 with MDM and its expression in skin suggests its role in maintaining the physiological and structural integrity of the epidermis.

In this study, we investigated a large consanguineous Pakistani family with 11 affected individuals with severe MDM to analyze genetic defect at the molecular level. After linkage to the ARS (component B) gene region with microsatellite marker D8S161 (Zmax 3.92), direct sequencing of all the three coding exons of genomic DNA was carried out. The sequencing revealed a pathogenic missense mutation in exon 3 at base pair position 256G>A (G86R). Nucleotide 256 in exon 3 seems to be mutation hotspot, as this mutation 256G>A has been previously reported in Pakistani, Palestinian and Taiwanese families to cause less severe MDM phenotype.[5,11] A different mutation 256G>C also introduces the same amino acid change i.e. G86R in a Turkish kindred causing MDM.[12]

Molecular characterization of rare monogenic disorders like MDM in the inbred Pakistani population could be the best strategy to control affected births through carrier screening, genetic counseling and prenatal diagnosis. Further molecular analysis of mutational spectrum in ARS gene in Pakistani families with MDM will lead to determine the specific mutations among Pakistani patients with MDM for disease control.

ACKNOWLEDGEMENT

We are grateful to all members of the family who participated in this study. Thanks to the dermatologist for clinical evaluation of the patients. This research was financially supported by the HEC indigenous PhD program and the Swedish Research Council (Swedish Links Asia to Shahid Mahmood Baig and Niklas Dahl) collaborative Research grant.

REFERENCES

1. Bouadjar B, Benmazouzia S, Prud'homme JF, Cure S, Fischer J. Clinical and genetic studies of 3 large, consanguineous, Algerian families with Mal de Meleda. Arch Dermatol 2000;136:1247-1252.
2. Patel H, Nardelli M, Fenn T, Houlston R, Coonar A, Patton MA, et al. Homozygosity at chromosome 8 qter in individuals affected by mal de Meleda (Meleda disease) originating from the island of Meleda. Br J Dermatol 2001;144:731-734.
3. Frenk E, Guggisberg D, Mevorah B, Hohl D. Meleda disease: Report of two cases investigated by electron microscopy. Dermatology 1996;193:358-361.
4. Fischer J, Bouadjar B, Heilig R, Huber M, Lefèvre C, Jobard F, et al. Mutations in the gene encoding SLURP-1 in mal de Meleda. Hum Mol Genet 2001;10:875-880.
5. Eckl KM, Stevens HP, Lestringant GG, Westenberger-Treumann M, Traupe H, Hinz B, et al. Mal de Meleda (MDM) caused by mutations in the gene for SLURP-1 in patients from Germany, Turkey, Palestine and United Arab Emirates. Hum Genet 2003;112:50-56.
6. Chimienti F, Hogg RC, Plantard L, Lehmann C, Brakch N, Fischer J, et al. Identification of SLURP-1 as an epidermal neuromodulator explains the clinical phenotype of Mal de Meleda. Hum Mol Genet 2003;12:3017-3024.
7. Cottingham R, Idury RM, Schaffer AA. Faster sequential genetic linkage, computations. Am J Hum Genet 1993;53:252-263.
8. Muslumanoglu MH, Saracoglu N, Cilingir O, Basmaci T, Urer S, Sabuncu I, et al. A novel mutation in the ARS (component B) gene encoding SLURP-1 in a Turkish family with mal de Meleda. Br J Dermatol 2006;155:467-469.
9. Fischer J, Bouadjar B, Heilig R, Fizames C, Prud'homme JF, Weissenbach J. Genetic linkage of Meleda disease to chromosome 8qter. Eur J Hum Genet 1998;6:542-547.
10. Mastrangeli R, Donini S, Kelton CA, He C, Bressan A, Milazzo F, et al. ARS component B: structural characterization, tissue expression and regulation of the gene and protein (SLURP-1) associated with Mal de Meleda. Eur J Dermatol 2003;13:560-570.
11. Wajid M, Kurban M, Shimomura Y, Christiano AM. Mutation in the SLURP-1 gene underlie Mal de Meleda in three Pakistani families. J Derm Sci 2009;56:27-32.
12. Chao SC, Huang CY, Lai FJ, Yang MH. Pseudominant inheritance with the G86R mutation in the ARS gene in Mal de Meleda. Int J Dermatol 2006;45:1458-1461.

Authors:

1. Sadia Nawaz,
2. Muhammad Tariq,
3. Aysha Azhar,
4. Mahmood Rasool,
5. Syeda Marriam Bakhtiar,
6. Ilyas Ahmad,
7. Shoaib ur Rehman,
8. Muhammad Jameel,
9. Tahir Naeem Khan,
10. Shehla Anjum Baig,
11. Joakim Klar,
12. Niklas Dahl,
13. Shahid Mahmood Baig·

1-9,13: Human Molecular Genetics Laboratory,
Health Biotechnology Division,
National Institute for Biotechnology and
Genetic Engineering (NIBGE),
Faisalabad, Pakistan.

10: Mother Child Health Center (MCHC),
Children's Hospital,
Pakistan Institute of Medical Sciences (PIMS),
G-8/3, Islamabad, Pakistan.

11, 12: Department of Genetics and Pathology,
The Rudbeck Laboratory,
Uppsala University,
SE-751 85 Uppsala,
Sweden.