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Recommended Citation

Naz, N., Samdani, A. J. (2011). Detection of filaggrin gene mutation (2282del4) in Pakistani Ichthyosis vulgaris families.. *JCPSP: Journal of the College of Physicians and Surgeons--Pakistan*, 21(6), 382-383.

Available at: http://ecommons.aku.edu/pakistan_fhs_mc_med_med/425

Detection of Filaggrin Gene Mutation (2282del4) in Pakistani Ichthyosis Vulgaris Families

Naghma Naz¹ and Azam Jah Samdani²

ABSTRACT

The aim of this study was to detect an 811 bp filaggrin (FLG) gene fragment known to carry a mutation 2282del4 which causes ichthyosis vulgaris. Seven clinically examined ichthyosis vulgaris families were included in this study. An 811 bp FLG gene fragment was targeted in the genomic DNA of all the members of the seven families by PCR amplification using known primers RPT1P7 and RPT2P1. Successful amplification of an 811 bp FLG gene fragment in all the families suggested the possible role of the 2282del4 mutation in causing ichthyosis vulgaris in Pakistani population.

Key words: *Ichthyosis vulgaris. Filaggrin gene. 2282del4 mutation. PCR amplification.*

Ichthyosis vulgaris is an inherited skin disease in which the patients show an abnormal skin cornification and generalized fine scaling. The other important clinical features include more intense scaling on the extensor surfaces of the extremities of the lower abdomen, arms and legs, hyperkeratosis and hyperlinearity of the palms and soles, atopic dermatitis, asthma, allergic rhinoconjunctivitis, hypohydrosis, and keratosis pilaris.¹ A defective processing of filaggrin in ichthyosis vulgaris patients has been demonstrated by biochemical studies. Filaggrin is a filament aggregating protein formed by the proteolytic cleavage of the precursor profilaggrin during terminal differentiation of the granular cells. The protein plays an important role in the formation of stratum corneum which forms the outermost barrier layer of the skin and provides protection against water loss, allergens and infectious agents.²

Genetic studies have revealed a number of mutations in the filaggrin (FLG) gene as being the cause of ichthyosis vulgaris in different populations.^{3,4} The aim of this study was to target and amplify an 811 bp FLG gene fragment in seven Pakistani ichthyosis vulgaris families.

This observational study was carried out at The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi and Department of Dermatology, Jinnah Postgraduate Medical Centre (JPMC), Karachi. Ethical approval for this study was obtained from the Research Ethics Committee of

KIBGE. A total of 16 affected (patient) and 19 unaffected (control) members belonging to seven unrelated families, having the family history of ichthyosis vulgaris were included in this study. The ichthyosis vulgaris families were given the codes IV1, IV2, IV3, IV4, IV5, IV6 and IV7. Pedigrees and other details of the families such as the inclusion and exclusion criteria have previously been published.⁵ Genomic DNA from the blood samples (500 µl) was prepared using FlexiGene DNA preparation kit (QIAGEN) according to the manufacturer's guidelines. DNA storage was carried out at -20°C. An 811 bp PCR fragment was amplified using the genomic DNA as the template. The primers used for the amplification were RPT1P7 (5' – AAT AGG TCT GGA CAC TCA GGT – 3') and RPT2P1 (5' – GGG AGG ACT CAG ACT GTT T – 3'). Amplification was carried out in a total volume of 50 µl containing 1 x PCR buffer with added 1.5 mmol MgCl₂, 5 mmol of each primer, 0.2 mmol of each of the four deoxynucleotide triphosphates (dNTPs), 10 ng genomic DNA and 0.5 U DNA Polymerase (KOD XL DNA Polymerase, Novagen). The amplification conditions were as follows: (94°C 5 minutes) x 1 cycle; (94°C 30 seconds, 57°C 45 seconds, 72°C 1.5 minutes) x 35 cycles; and a final extension at 72°C for 5 minutes. Electrophoresis of the amplified DNA (10 µl) was carried out on 1% agarose gels at 80 V using TAE buffer (40 mM Tris-acetate, 5 mM sodium acetate, 0.1 mM Na₂-EDTA, pH-7.8). The gels were observed and analyzed by using a gel documentation system. Amplification of the 811 bp DNA fragment was found successful in all the seven families included in this study (Figure 1).

The genetic defects responsible for causing ichthyosis vulgaris have been mapped in the exon 3 of the FLG gene which codes for filaggrin protein. Among these defects, mutations including R501X, 2282del4, 3321delA and 3702delG have been studied in American, Japanese and European populations.^{3,4} In our previous study, the presence of a 1.5 kb FLG gene fragment known to carry

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Received May 07, 2010; accepted March 25, 2011

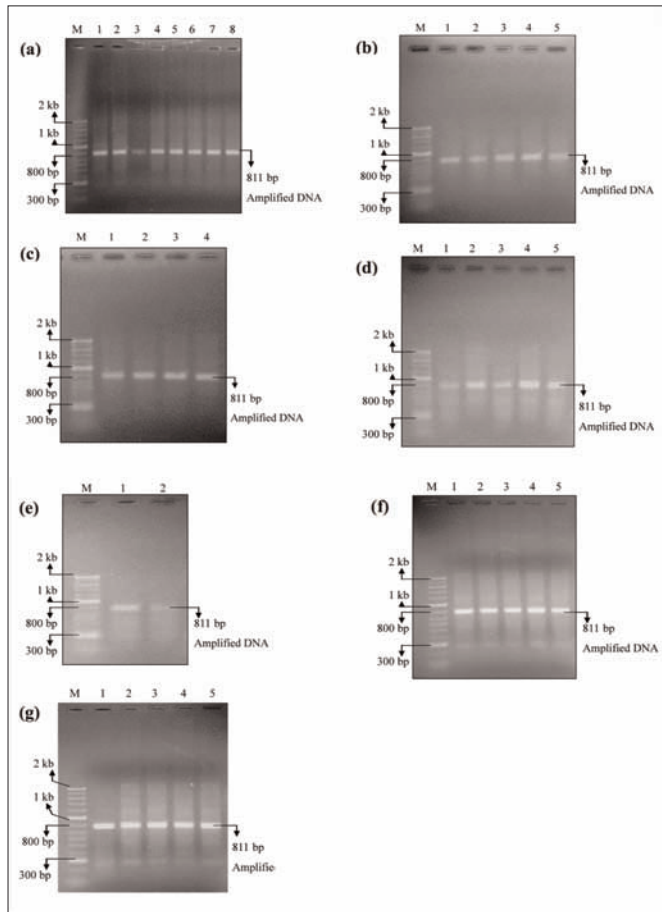


Figure 1: PCR amplification of 811 bp FLG gene fragment in Pakistani ichthyosis vulgaris families: IV1 (a), IV2 (b), IV3 (c), IV4 (d), IV5 (e), IV6 (f) and IV7 (g); Numbered lanes: amplified DNA; M: molecular weight marker (Bioline - Hyperladder II).

R501X mutation has been reported in five out of the seven Pakistani ichthyosis vulgaris families included in this study.⁵ In the current study, amplification of an 811 bp FLG fragment known to carry the 2282del4 mutation was carried out using the known primers which have previously been used for the amplification of 811 bp FLG gene fragment with 2282del4 mutation in other populations.³ During this study, successful amplification of the 811 bp FLG gene fragment suggested the possibility

of the presence of the 2282del4 mutation in Pakistani ichthyosis vulgaris patients (Figure 1). The results obtained during this study also suggested the possible role of the 2282del4 mutation in addition to the R501X mutation⁵ as being one of the major genetic causes of the disease in our population.

Many recessive disease genes, which otherwise remain inactive, express in disease phenotype when unite as a result of extensive inbreeding within a family. In Pakistan, consanguineous marriages are common, as a result of which disease genes keep on passing from one generation to another and thus increasing the spread of the inherited diseases. It is important to study the genetic basis of diseases to provide genetic counselling and awareness to people. This leads to the better health of the population in general. The results of the current study will provide useful knowledge for further research in the field of the molecular basis of ichthyosis vulgaris in Pakistan.

Acknowledgement: This project was funded by the HEC (Higher Education Commission), Pakistan.

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