A Novel Large Deletion in the *EVER1* Gene in a Family With Epidermodysplasia Verruciformis From India

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Abstract: Epidermodysplasia verruciformis (EV) is a rare autosomal recessive genodermatosis due to mutations in *EVER1* and *EVER2* genes. The genetic profile of Indian patients with EV has not been previously studied. This report describes the clinical presentation and molecular analysis of a family with EV. Using genomic DNA from two affected probands and healthy controls (two other siblings), conventional polymerase chain reaction (PCR) was conducted with novel primer sets designed to amplify the coding and splice-site regions in the genes *EVER1* and *EVER 2*. This revealed no amplification with a primer set for exons 16 to 18 in the *EVER1* gene of both the probands. Subsequently, long-range PCR spanning the length of exon 15–20 and next-generation sequencing demonstrated a homozygous deletion of 2078 bp in the *EVER1* gene (*EVER1*:c.2072_2278del). Screening the family revealed the same homozygous deletion (similar to index cases) in two other affected siblings. The parents and two asymptomatic siblings were heterozygous carriers for the deletion while one healthy sibling was negative. These results were validated with Sanger sequencing. This deletion in exons 17 and 18 of the *EVER1* gene results in a frameshift, followed by a premature termination resulting in a severe phenotype. The identification and validation of this large deletion was detected using stepwise amplicon-based target enrichment and long-range PCR, respectively. In this family, this simple strategy greatly enhanced genetic counseling as well as early genetic diagnosis and screening. However, functional assays and larger studies are required to characterize and validate the genetic diversity among Indians with EV.

Key Words: genetic skin diseases, epidermodysplasia verruciformis, *EVER1* mutation, large deletion, rare diseases

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INTRODUCTION

Epidermodysplasia verruciformis (EV) is a rare autosomal recessive genodermatosis predisposing affected individuals to infections with human papilloma viruses (HPVs), thereby increasing the risk of non-melanoma skin cancer.[1] The majority of cases are inherited, classically with loss-of-function mutations at the *EVER1/TMC6* and *EVER2/TMC8* genes in as many as 75% of described cases.[2] The pathogenesis is linked to a loss of function of TMC6 and TMC8 proteins, which are coded by the *EVER1* and *EVER2* genes located on chromosome 17q25.[3,4] Within keratinocytes, these proteins (located on the endoplasmic reticulum) modulate the intracellular distribution of zinc and subsequently influence the activity of transcription factors necessary for HPV proliferation. In patients with EV, there is altered intracellular zinc homeostasis, increased HPV proliferation within the keratinocytes, and potential risk of malignant transformation.[5,6]

The diagnosis of EV is usually clinical, aided by histopathological evidence of HPV infection. Patients classically present with numerous polymorphic skin lesions, first appearing during infancy/childhood and then remaining throughout life. While mucous membranes are generally spared, the skin lesions include verruca, verruca plana, pityriasis versicolor, and seborrheic keratosis–like lesions.[7,8] The risk of squamous cell carcinoma (SCC) among affected individuals is reported to be as high as 66% with almost 100% incidence in the presence of high-risk skin lesions. The onset of non-melanoma skin cancer begins in adolescence and can persist into early adulthood.[7] Given the predisposition for malignancy, early genetic diagnosis may benefit both affected individuals and other family members alike, providing an opportunity for screening, early monitoring of suspicious skin lesions, and timely intervention.[9]

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The authors declare no conflicts of interest.

The data that support the findings of this study have been added to the manuscript.

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Although more than 500 cases have been reported worldwide, within India, limited information on the genetic alterations in affected individuals is available owing to the rarity of the disease.[1] A recent review by Parul et al described 20 Indian patients with a clinical diagnosis of EV. However, 80% of cases were sporadic, and mutational analysis was unavailable.[10] In this report, we describe the detection of a large deletion mutation in *EVER1* among affected members of a family, which also served as a screening tool for carriers.

Patient Data

A 34-year-old man (Patient 1) and his 28-year-old brother (Patient 2) presented with skin lesions all over the body since childhood. They were born of a consanguineous marriage and had seven other siblings. Among the nine children, three had similar lesions while the parents were unaffected. Both the patients had multiple hypopigmented macules resembling pityriasis versicolor over the face, trunk, and extremities and hyperpigmented thin flat plaques resembling seborrheic keratosis and verrucous papules over the face and scattered over the trunk. Based on the history and clinical features, a clinical diagnosis of EV was made (Fig. 1). Multiple biopsies performed from various suspicious lesions were positive for Bowen disease and actinic keratosis (Fig. 2). Patient 1 had an invasive SCC involving the presternal region and back for which he underwent wide local excision, and

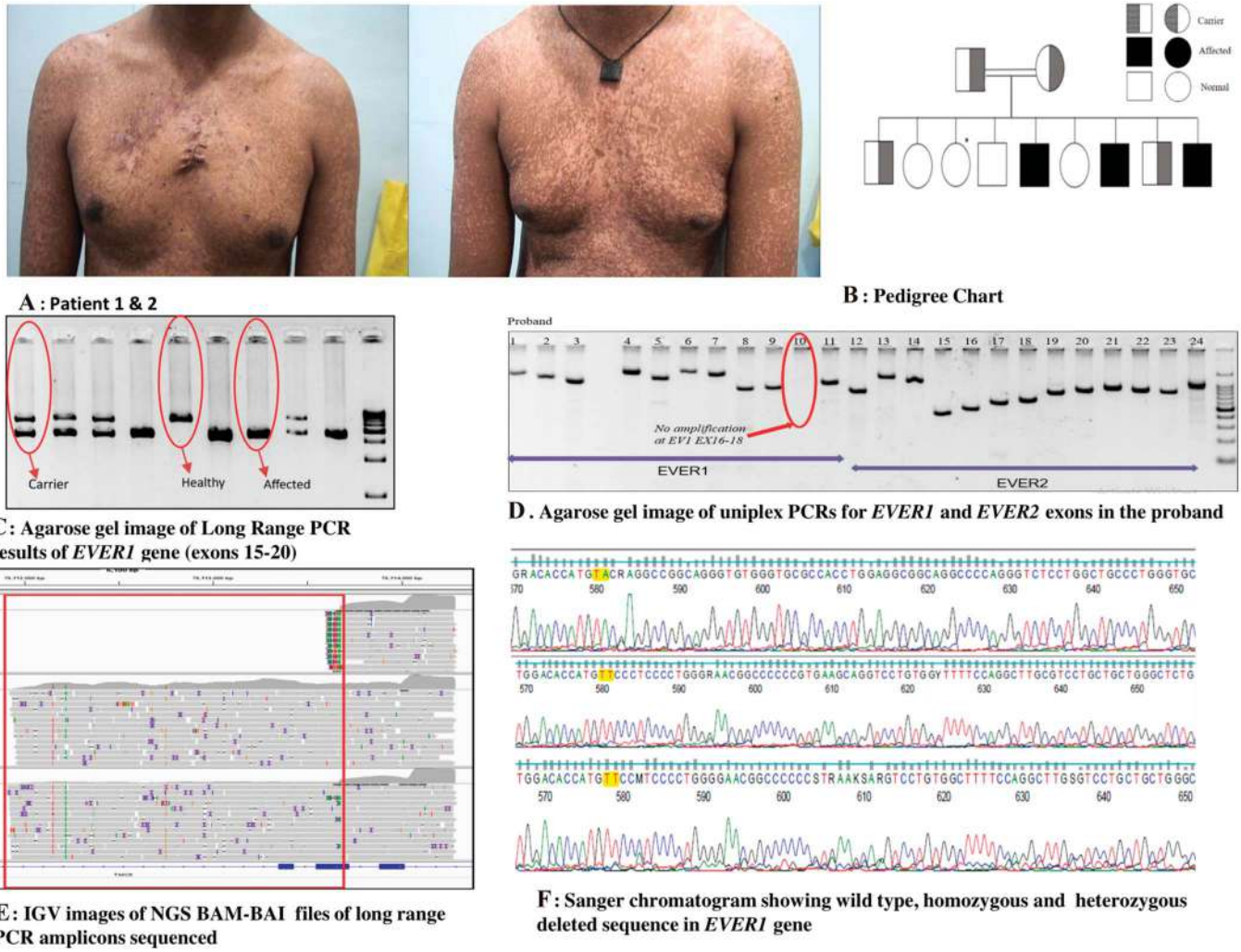

FIGURE 1. A, Patients 1 and 2: Images show numerous hypopigmented pityriasis versicolor–like and verruca plana–like lesions all over the body in both patients. Patient 1 bears a scar of wide local excision of an invasive SCC on the chest. B, Pedigree chart: *phenotype could not be determined because of the inability to perform a full physical examination owing to social constraints. C, Agarose gel image for uniplex PCRs showing amplification of *EVER 1* and *EVER 2* genes and the missing amplicon of exons 16–18 in the proband. D, Long-range PCR (*EVER1*—exons 15–20); product size: homozygous wild-type ~7 kbp, heterozygous deletion ~7 and 5 kbp, and homozygous deletion ~5 kbp. E, Integrated Genomics Viewer image on BAM—BAI file of (A) homozygous deletion extending from exon 17 to 18 with zero reads, (B) healthy normal negative for the deletion, and (C) heterozygous carrier for the deletion with read depth decreased by 60%. F, Sanger chromatogram showing wild-type, homozygous, and heterozygous deleted sequences from exon 17 to 18 in the *EVER1* gene.

Patient 2 had a well-defined SCC of the upper lip and invasive SCC over the arm and back. There were no features of metastasis in both of them, and they were initiated on oral acitretin.

MATERIALS AND METHODS

The molecular investigations were performed after obtaining informed consent from the patients. Using in-house designed novel primer sets, *EVER1* and *EVER2* gene target enrichment was performed using genomic DNA extracted from the blood samples of the affected probands (patients 1 and 2). Long-range polymerase chain reaction (PCR) was performed to confirm the extent of deletion. The molecular weight of the segments was demonstrated using Southern blot analysis. The products were subsequently sequenced by next-generation sequencing to delineate the exact deleted segment. Using previously published protocols, the long-range amplicons in the probands and controls were sheared, adapter ligated, and size selected, followed by clonal amplification with emulsion PCR and sequencing on Ion Torrent Personal Genome Machine.[11] The generated BAM files were then analyzed on an Integrated Genomics Viewer. Further validation was performed with conventional Sanger sequencing of this region.

RESULTS

The conventional PCR showed no amplification for the amplicon with primers covering the exons 16–18, whereas all the primer sets showed amplification with healthy controls. To detect any possible deletion, a long-range PCR with primer sets from exon 15 to exon 20 was designed, and the results were indicative of a large deletion of around 2000 bp (control 7 kbp, test 5 kbp). The carriers had product amplification with both the bands. Using next-generation sequencing, a homozygous deletion of 2078 bp spanning exons 17 and 18 was detected, and the *EVER1* deletion c.(2071_2198+126)_(2199_2278+1752) del was identified, confirming the diagnosis of EV. This deletion resulted in a frameshift (c.2070_2072) and a premature termination at codon 691 (TAC (Tyr) with TGA (Ter)) (Fig. 1). The samples of the parents and the other siblings were then assessed using the same methods. Two other affected siblings showed a similar pattern as that of the index cases. Both the parents and 2 asymptomatic siblings were heterozygous carriers for the deletion while the other siblings were homozygous negative.

DISCUSSION

Although the findings of this report are in harmony with the recessive nature of this disease, deletion mutations within *EVER1* and *EVER2* genes are uncommon.[12,13] More commonly, nonsense mutations, splice-site mutations, and frameshift mutations have been described internationally.[3,13,14] To the best of our knowledge, there is no published literature on the genetic profile of Indians with EV.

In this report, the extent of deletion in *EVER1*:c.2072_2278del resulted in a frameshift, followed by a premature termination at codon 689 instead of codon 806. This truncated protein product leads to a loss of function at the transmembrane domain of the protein and may affect zinc homeostasis, thus correlating with the phenotype. According to the ACMG 2015 guidelines, this mutation is classified as Pathogenic.[15] However, functional validation is required to further understand the pathogenicity of this deletion.

In our analysis, identification of this large deletion was solely achieved using stepwise amplicon-based target enrichment and validation with long-range PCR. Sequencing was subsequently performed to characterize the deleted segment. In instances of high clinical suspicion, this simple strategy could greatly enhance genetic counseling as well as the early genetic diagnosis and screening within affected families.

CONCLUSIONS

Deletion mutations implicated in the pathogenesis of EV are uncommon and require functional validation.

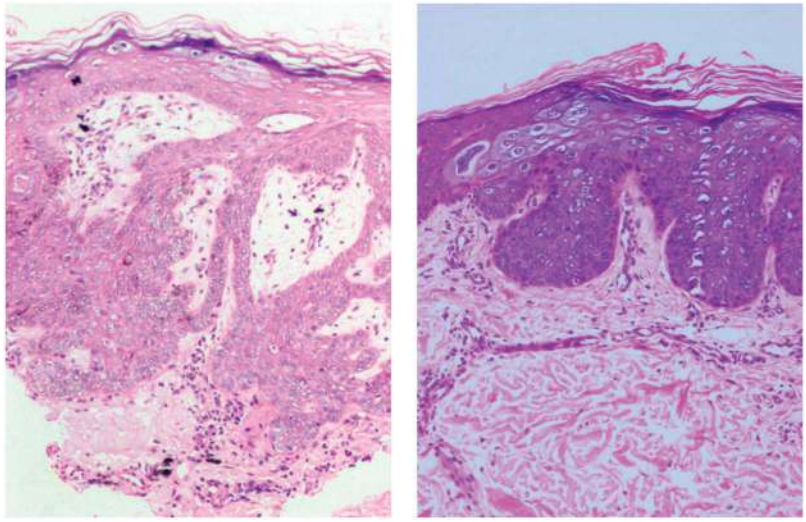

FIGURE 2. Left: Photomicrograph showing the section of skin with epidermis displaying large cells with gray–blue cytoplasm in the spinous and granular layer and full-thickness epidermal dysplasia with a intact basement membrane and loss of polarity with moderately pleomorphic nuclei (H&E ×100). Right: Photomicrograph shows the section of skin with epidermis displaying large cells with gray–blue cytoplasm in the spinous and granular layer and infiltrated by a tumor with interwoven trabeculae and anastomosing cord malignant squamous cells extending to a depth of 1 mm (H&E ×100).

Understanding the genetic profile of these patients significantly improves genetic counseling and early diagnosis. There is a need for further molecular research among patients with EV in India. The rare nature of the disease underscores the importance of larger, perhaps multicentric studies to understand the genetic diversity within this disease.

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