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Novel mutations of *EVER1/TMC6* gene in a Japanese patient with epidermodysplasia verruciformis

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Abstract Germline mutations of the *EVER1/TMC6* gene are associated with epidermodysplasia verruciformis (EV), which is characterized by an abnormal susceptibility to human papillomaviruses that were considered to be innocuous for the general population. In this study, we have employed polymerase chain reaction and DNA sequencing analysis to characterize the *EVER1* gene in a 65-year-old Japanese EV patient. Direct sequence analyses resulted in the identification of two novel mutations. One nonsense mutation consisting of a (C>A) transversion at nucleotide 744 in exon 8 in one *EVER1* allele resulted in the introduction of a premature termination codon (Y248X). Another mutation was identified in the splice acceptor site of intron 8 (892-2, IVS8-2, A>T) in another allele. This is the second report of *EVER1/TMC6* mutations in EV.

Keywords Epidermodysplasia verruciformis · *EVER1/TMC6* · Mutation

Introduction

Epidermodysplasia verruciformis (EV) (OMIM#226400) is a rare autosomal recessive disorder characterized by an abnormal susceptibility to infection by specific human papillomaviruses (HPVs) and an apparently increased risk of skin cancer. Carcinomas develop particularly in sun-exposed skin and are usually associated with HPV5 or HPV8. EV thus represents an unusual condition in which HPV infection, inherited predisposition, and exposure to the sun all play a part and a model system, which provides us an opportunity

to analyze the genetic basis for the susceptibility to HPV infections (Lutzner and Blanchet-Bardon 1987). Ramoz et al. (2002) studied two Algerian and two Colombian consanguineous EV families and identified that *EVER1/TMC6* and *EVER2/TMC8* genes are responsible for the EV. *EVER1* (19 exons) and *EVER2* (15 exons) genes are in the opposite orientation from the first in frame initiation codon and are separated by 4,732 bp. Two kinds of *EVER1* transcripts, 2.8 Kb in full length and 1.7 Kb that skipped from exon 11 to exon 18, are reported (Ramoz et al. 2002; Keresztes et al. 2003; Kurima et al. 2003). The gene product, EVER1, has features of integral membrane proteins and is localized in the endoplasmic reticulum (Ramoz et al. 2002). Here we report novel *EVER1* mutations in an EV patient.

Case report

The patient was a 65-year-old Japanese woman. She frequently suffered from flat warts beginning when she was a teenager. Excisions of Bowen disease in the hand were carried out three times when she was 51, 52, and 64, and a gastrectomy was performed because of gastric cancer when she was 53. These surgeries were performed at another hospital. Diagnosis of EV was made when she was in her twenties. Surgery was carried out at this hospital because multiple skin cancers were found on her face. Histological diagnosis of these excised skin cancers showed squamous cell carcinoma and Bowen disease (data not shown). Her parents had died of heart disease and showed no sign of EV. They were not consanguineous. Three individuals are affected with EV among her five brothers and one sister. The family history was taken from the proband only, and the authors did not examine it. Informed consent for genetic analysis was obtained from the patient and normal individuals. However, genetic analyses of her brothers and her daughter were unavailable because they refused to provide the blood.

Methods

Genomic DNA was prepared from peripheral blood leukocytes by standard methods using phenol/chloroform extraction and ethanol precipitation. The exons and the intron/exon boundaries in the *EVER1* gene were analyzed by polymerase chain reaction (PCR)

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and direct sequencing of the PCR products (Tate et al. 2003). The primers used for amplification of the genomic DNA were as follows: EVER1, ex8-1, 5'-TGG CGC TAC GCC CTG AAG CGC ATC-3', EVER1, ex8R, 5'-GTA CCA GGG TAG CAG AAG ACA GG-3', EVER1, ex9F, 5'-CGT GGG GAA AGT CCC TGA CTC GGC-3', EVER1, ex9R, 5'-GAG TGG AGC TCG GAG CCT GGG TC-3'. Primer sequences for other exons are available on request. Amplified DNA fragments were recovered from a low-melting-temperature agarose gel and used for the sequencing analysis. We sequenced the PCR products directly by using a DNA sequencing system (model 377; Applied Biosystems). The direct sequencing was performed in both directions, and the mutation analysis was repeated independently. Nucleotide numbering is based on Genbank sequence AY099356 (*EVER1* 2.8 Kb cDNA), AY099357 (*EVER1* 1.7 Kb cDNA) and NT010641 (*EVER1* genomic DNA; gi: 29797789). PCR fragments amplified by a pair of primers, EVER1, ex8-1 and ex9R were cloned by pGEM-T easy vector systems according to the manufacturer's technical protocol (Promega, Madison, WI, USA) and sequenced by primer extension methods.

Results

As shown in Fig. 1, mutational analysis of the *EVER1* gene using primers EVER1, ex8-1, and ex9R revealed a heterozygous single nucleotide transversion from C to A at nucleotide 744, which resulted in the introduction of a premature termination codon in exon 8 (Y248X). In addition, we found another heterozygous single nucleotide transversion in the splice acceptor site of intron 8 (892-2, IVS8-2, A > T) (Fig. 2). Taken together with the

Fig. 1 A nonsense mutation in exon 8 of the *EVER1* gene in the epidermodysplasia verruciformis (EV) patient. Genomic DNA sequence analysis of exon 8 revealed a heterozygous transversion at nucleotide 744 in exon 8, which is shown by *N*, resulting in a premature termination codon (744 C > A). *N* indicates the overlapping waves G and T. The reverse-complementary nucleotide sequence is shown

first report that described that *EVER1* is the responsible gene of EV (Ramos et al. 2002), sequencing data obtained from 50 normal individuals showed no nucleotide transversions in these nucleotides (data not shown), indicating that these two transversions are not polymorphisms but mutations.

To determine whether two mutations found in this study locate on the same allele or separately on the different alleles, PCR fragments amplified with a pair of primers EVER1, ex8-1 and EVER1, ex9R were cloned with pGEM-T easy vector and sequenced. The results of the sequence by primer extension indicated that two mutations located on the different alleles, consistent with the autosomal recessive trait (data not shown).

Discussion

Four kinds of mutations in the *EVER1* and *EVER2* genes have already been reported in EV patients of two Algerian and two Colombian consanguineous families (Ramos et al. 2002). As shown in Table 1, three of four *EVER1* mutations are nonsense mutations, and one is a splicing site mutation. Carcinomas develop particularly in sun-exposed skin and are usually associated with HPV5 or HPV8 in EV. In this study, however, E6 genes of HPV5 and HPV8 were undetected in the skin cancer

Fig. 2 A splicing mutation in intron 8 of the *EVER1* gene in the epidermodysplasia verruciformis (EV) patient. Genomic DNA sequence analysis at the splicing acceptor site of intron 8 revealed a heterozygous A to T substitution (892-2, IVS8-2 A > T). *N* indicates the overlapping waves A and T. The nucleotide A occurs in the wild-type sequence and the nucleotide T in the mutated sequence. The reverse-complementary nucleotide sequences and amino acid numbers are shown

Table 1 Summary of *EVER1* mutations in EV

Mutation	Splice mutation	Splice site	Exon	IVS ^a	Amino acid	Source
280 (C > T) ^b	No		5		R94X	Ramos et al. (2002)
744 (C > A)	No		8		Y248X	Present study
892-2 (A > T)	Yes	Acceptor		8		Present study
1726 (G > T)	No		14		E576X	Ramos et al. (2002)

^aIVS, intervening sequence

^bNucleotide position 1 is the A in the ATG translation initiation codon in the *EVER1* cDNA sequence

by PCR (data not shown). An EV patient in this case suffered from gastric cancer in addition to skin cancer. Her brother with EV also suffered from gastric cancer, and her sister with EV suffered from bilateral breast cancer, indicating that *EVER1* mutations may be involved in cancer predisposition not only in skin but also in other organs.

The function of the *EVER1* molecule is unknown, although it is reported that *EVER1* is localized in the endoplasmic reticulum (Ramos et al. 2002). Therefore, it remains to be discovered why HPV infection caused skin cancer in the EV patient and which steps, such as antigen presentations against HPV, cytokine secretions, or cytotoxic T-lymphocyte inductions, are disturbed. In conclusion, we report here the first *EVER1* mutation in a Japanese patient with EV.

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