Analysis of TYR Gene Pathogenic Variants in a Chinese Mongolian Family with Progressive Symmetric Erythrokeratoderma

Abstract

This study sought to analyse tyrosinase (TYR) pathogenic variants in a Chinese Mongolian family with progressive symmetric erythrokeratoderma (PSEK). We collected clinical data and peripheral blood DNA samples from the initial patient and his family members for polymerase chain reaction (PCR) amplification and whole-exome sequencing of the coding region of TYR. Genetic analysis showed a TYR insertion (c. 929_930insC; p.Arg311Lysfs*7) in the patient that was not detected in any of the normal family members or in 100 healthy controls. This report provides the first description of this TYR pathogenic variant (c. 929_930insC) in a family; functional studies and further research are needed for an in-depth analysis.

Keywords: *Gene pathogenic variant, progressive symmetric erythrokeratoderma, TYR*

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Introduction

PSEK, a rare genetic keratotic skin disorder, is usually autosomal dominant but in some cases is autosomal recessive.[1] PSEK is characterised by early onset and slow progression. Symmetric erythema is mainly located on the extensor side of the extremities, with or without palmar-plantar keratosis.[2] Researchers at home and abroad have reached different conclusions about the causative genes of PSEK, with the following genes implicated: Loricrin (LOR),[3] gap junction β4 (GJB4),[4] gap junction β2 (GJB2),[5] keratin 83 (KRT83),[1] 3-ketodihydrosphingosine reductase (KDSR),[6] and transient receptor potential melastatin 4 (TRPM4).[7] We collected blood samples from a Chinese Mongolian family with PSEK and performed whole-exome sequencing to detect gene pathogenic variants. The results are reported herein.

Materials and Methods

Clinical data

The family consists of 18 members from four generations [Figure 1], including 1 patient and 17 normal individuals (male: 8, female: 9). The priori-proband (III-6) is a 23-year-old male (ethnicity: Mongolian). The disorder was evident at birth, manifesting as bilateral palmar-plantar rough skin with mild scaling. The disorder was not addressed, and the patient did not receive any treatment. As the patient grew older, scaling worsened, and symmetric keratinized erythema with clear boundaries gradually occurred on the bilateral palms, back of the hands, sole of the feet, and back of the feet, with mild infiltration and hypertrophy. The lesions later spread to the bilateral wrists and ankles, with no ulceration, exudation, or pain. His hair, teeth, fingernails, hearing, and mucous membranes were normal, and his big toenails were partially yellow. Lesions alleviated on their own and were recurrent, and the symptoms were worse in winter than in summer. The patient had no family history of this condition. His parents were unrelated by at least three generations. The patient was born full-term, and his twin elder sister (III-5) had no such skin conditions. Physical examination showed normal development, a generally good condition, and normal organs and systems. Dermatology examination showed symmetric keratinized erythema with clear boundaries on the bilateral palms, back of the hands, wrists, sole of the feet, back of the feet, and ankles, with mild infiltration, hypertrophy, and scaling as

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well as partial yellowing of bilateral big toenails [Figure 2]. Laboratory tests showed normal blood results, urine analysis results, and liver and kidney function, and negative fungal culture and microscopic examination of the bilateral big toenails. Biopsy of the lesion on the back of his left foot showed hyperkeratosis, hypertrophy of the granular layer and spinous layer, an intact basal layer, and superficial dermal perivascular infiltration of a small amount of inflammatory cells [Figure 2]. PSEK was diagnosed based on typical clinical manifestations and pathological findings. The patient was given topical isotretinoin ointment alternating with cod liver oil ointment, twice a day for 2 months. Moreover, the patient was instructed to cover the lesions with plastic wrap for 15-20 minutes after each application. The lesions alleviated; however, the patient reported that the symptoms recurred after treatment discontinuation for 2 weeks and then declined any further treatment.

Methods

Genomic DNA extraction from peripheral blood

After obtaining informed consent from each family member included in this study, a venous blood sample (3-5 mL) was collected from the PSEK patient, each family member, and each healthy control into a negative-pressure EDTA-K_3- or EDTA-Na_4-treated tube. The samples were labelled with the subject ID and basic information and then stored at -80°C for later analysis. A DNA extraction kit from Shenzhen Huada Gene Technology, Co., Ltd. was used to extract genomic DNA.

PCR and sequencing

Genome sequences including exons, introns, 5'UTR Exons and 3'UTRExons were input into Primer 5.0 software, and primers were designed for the exon coding region of TYR and the region 50-100 BP beyond the target sequence boundary. Reference primer sequences and PCR reaction conditions were used to amplify all exons of TYR gene. The PCR products were obtained by agarose gel electrophoresis detection. The purified PCR products were placed in CEQ8800 sequencer for sequencing.

Results

The PCR amplified products were qualified by agarose gel electrophoresis and verified by Sanger sequencing, Confirm the patient (III-6) had a TYR insertion (c. 929_930insC; p.Arg311Lysfs*7) that resulted in an insertion of C between loci 929 and 930 of TYR. Consequently, arginine (Arg) at position 311 was replaced by lysine (Lys), along with a frameshift pathogenic variant, resulting in a new stop codon at position 7. None of the normal family members or the 100 healthy controls carried this pathogenic variant, which is consistent with the pattern of family segregation. In summary, we confirm that a TYR pathogenic variant (c. 929_930insC) was associated with PSEK in this family [Figure 3].
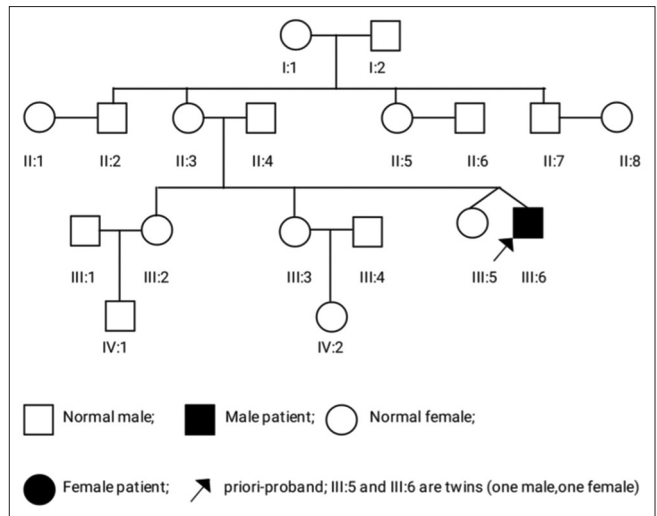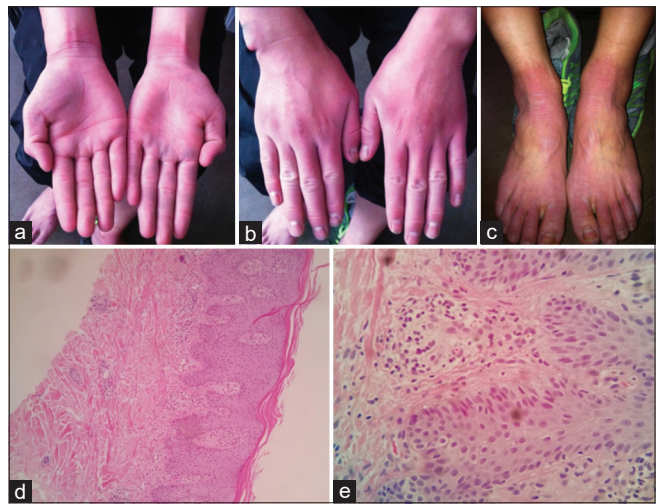

Figure 1: PSEK pedigree

Figure 2: Clinical symptoms of the priori-proband: (a) and (b): Symmetric keratinized erythema with clear boundaries on the bilateral palms, back of the hands, and wrists; (c): Symmetric keratinized erythema with clear boundaries on the bilateral back of the feet and ankles, with mild infiltration, hypertrophy, and scaling as well as partial yellowing of the bilateral big toenails; (d) and (e): Lesion biopsy (HE ×100, ×400) shows hyperkeratosis, hypertrophy of the granular layer and spinous layer, intact basal layer, and superficial dermal perivascular infiltration of a small amount of inflammatory cells
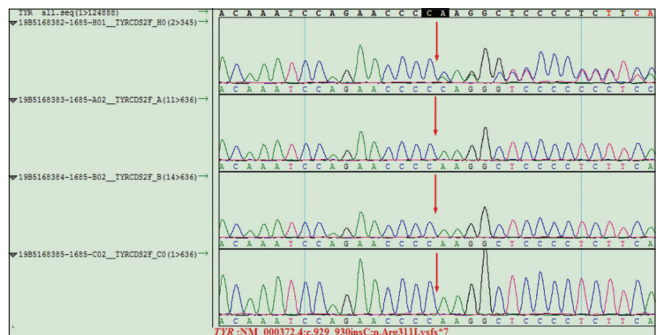

Figure 3: C is inserted between loci 929 and 930 of TYR. Consequently, arginine (Arg) at position 311 is replaced by lysine (Lys), resulting in a frameshift pathogenic variant new stop codon at position 7

Discussion

Since PSEK was first reported in 1911, more than 40 familial cases and 160 sporadic cases have been reported worldwide through 2019. In 1997, Ishida-Yamamoto *et al.*[3] detected a frameshift pathogenic variant (709insC) in *LOR* located on chromosome 1q21 in a Japanese family with PSEK and concluded that *LOR* was the causative gene of PSEK. In 2000, Richard *et al.*[8] analysed six families with PSEK from Switzerland, the UK, and the US. They did not detect any *GJB3* pathogenic variants, suggesting that *GJB3* pathogenic variants were unrelated to PSEK. It was suspected that the Japanese family reported in 1997 probably had keratoderma hereditaria mutilans (also known as Vohwinkel syndrome) rather than PSEK. However, in 2000, Suga *et al.*[9] established *LOR* defect-related keratosis associated with human PSEK and Vohwinkel syndrome in transgenic mice, thereby, validating the conclusion by Ishida-Yamamoto *et al.*[3] in 1997. In 2006, Chinese researchers Cui *et al.*[10] analysed a Chinese family with PSEK that included 7 generations and 16 patients. The results excluded *LOR*, *GJB3*, and *GJB4* as the causative genes of PSEK. The researchers performed whole-genome linkage analysis and located the causative gene on chromosome 21q11.2-21.2. To date, however, no specific causative gene has been identified in this region. In 2009, van Steensel *et al.*[4] detected a *GJB4* missense pathogenic variant in two PSEK patients via direct sequencing, suggesting that PSEK and erythrokeratoderma variabilis (EKV) may be related to *GJB4* pathogenic variants and represent different stages of the same disease spectrum. In 2017, Yang *et al.*[5] detected a *GJB2* missense pathogenic variant at locus 608 in a sporadic case of PSEK in China but did not detect any causative pathogenic variants in *GJB3*, *GJB4*, *GJB6*, *GJAl*, or *SERPINB7*. In 2017, Shah *et al.*[1] analysed a Pakistani family with PSEK and detected, with exon sequencing, a homozygous deletion (c. 811 delA; p.Ser271fs) and a frameshift pathogenic variant in *KRT83*, indicating autosomal recessive inheritance. In 2017, Boyden *et al.*[6] analysed 4 sporadic cases of PSEK in the US and detected three different *KDSR* deletions (c. 164_166delAAG, p.Gln55_Gly56delinsArg; c. 256_ 2A>C, p.Val86_Gln107del; and c. 879G>A, p.Gln260_Gln293del) and a missense pathogenic variant (c. 557A>T; p.Tyr186Phe). In 2019, Wang *et al.*[7] analysed two Chinese families with PSEK and a sporadic PSEK case and detected two missense pathogenic variants (c. 3099C>Gm c. 3119T>C) in *TRPM4*, an important player in the skin TRP pathway and a potential target for the treatment of hyperkeratosis. In 2019, French researchers Dereure[11] expanded the findings by Wang *et al.*[7] and demonstrated, with *in vitro* electrophysiological studies, that TRPM4 may be an important target for the treatment of hyperkeratotic dermatopathy. In 2016, we[12] analysed a Chinese Han family with PSEK and did not detect any *LOR* pathogenic variants.

In this study, we analysed a Chinese Mongolian family with PSEK and detected, via whole-exon sequencing, a TYR insertion (c. 929_930insC; p.Arg311Lysfs*7), indicating that a C was inserted between loci 929 and 930 of TYR. Consequently, Arg at position 311 was replaced by Lys, resulting in a frameshift pathogenic variant and a new stop codon at position 7. TYR is a member of the tyrosinase-related protein family.[13] This gene is located on chromosome 11q14-q21 and has a total length of 65 kb. It contains 5 exons and 4 introns and encodes a 58-kDa glycoprotein composed of 529 amino acids. Its product, TYR, is the rate-limiting enzyme in the process of melanin synthesis. TYR pathogenic variants are often reported for 2-allele diseases such as Waardenburg syndrome with albinism, oculocutaneous albinism type 1B, and oculocutaneous albinism type 1A. We will conduct functional studies in the future. It is necessary to expand the sample size of disease to further study the pathogenic genes and clinical symptoms of PSEK in Chinese population. Due to the environment, lifestyle, and unique customs, genetic drift is less common in Mongolian pedigrees, making these pedigrees valuable resources for investigating special diseases. In this study, we detected a new *PSEK* pathogenic variant in this Mongolian family, which further enriches our knowledge about the clinical and genetic characteristics of PSEK and the international gene pathogenic variant spectrum.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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